MANUAL ON VISCERAL LEISHMANIASIS (KALA-AZAR) IN INDIA





GOVERNMENT OF INDIA

NATIONAL MALARIA ERADICATION PROGRAMME

(DIRECTORATE GENERAL OF HEALTH SERVICES)

MINISTRY OF HEALTH & FAMILY WELFARE,

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GOVERNMENT OF INDIA

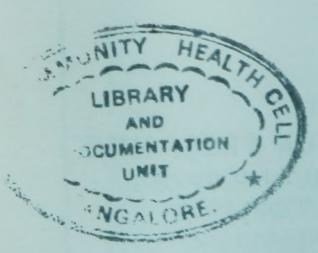
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PREFACE

Leishmaniases are well known in India for centuries and Bengal is the oldest known endemic area for Visceral Leishmaniasis (VL/kala-azar). Periodic out breaks/epidemics of kala-azar (VL) used to occur in India particularly in the eastern half of the country which used to last for about 10 years with inter epidemic period of 10-15 years during pre DDT era.

Advent of DDT and its use under National Malaria Control Programme (NMCP) since 1953 followed by National Malaria Eradication Programme (NMEP) since 1958, paid unexpected dividend to the VL problem, though the use was never aimed at VL control. The impact of spray coupled with effective treatment of VL cases was so dramatic that by sixties, the fresh kala- azar cases were no more recorded in most of the hospitals in erstwhile endemic areas of the country.

This virtual elimination of the disease gave an impression, though false, of elimination of the disease and the manufacturers of anti kala-azar drugs either scaled down or stopped the production. One of the most important drug, Urea Stibamine has become a history since then, as the unit manufacturing this drug was disbanded. Consequent upon withdrawl of NMEP activities in the erstwhile kala-azar endemic areas because of effective malaria control, in fact, removed the insecticidal pressure barrier from kala-azar vector population which started building up slowly. This building up, coupled with continued presence of Post Kala-Azar Dermal Leishmaniasis (PKDL) cases led to kala-azar resurgence initially in 4 districts of north Bihar in 70's with gradual spread. Though the control programme was launched in 4 districts of Bihar from 1977 to

1979 under overall monitoring and guidance of National Institute of Communicable Diseases (NICD), other areas were ignored and the disease continued to remain in the community with gradual spread. The control efforts initiated in 4 districts were also withdrawn within a short spell of 3 years even before the disease could be brought down to a level where it would have lost its continuance as a public health problem. This limited area operation and consequent withdrawal did not lead to an effective control of disease. The disease started increasing and spreading from 1980 onwards.

In view of the rising trend of kala-azar in India along with its gradual spread, Planning Commission approved a separate Kala-azar Control Scheme during the year 1990-91 which was further intensified from 1992. Until then, states attempted to tackle the problem on an adhoc basis by using insecticides diverted from NMEP for focal spraying which was not at all effective in restricting the spread and was only giving a limited, temporary and focalized impact. The VL control efforts in India from 1992 have given dramatic results with a decline of 67.37 % in morbidity and 73.2 % in mortality within two years of intensified programme implementation in Bihar and has reassured the probability of eradication of disease if the concerted efforts are continued.

Directorate of NMEP, as the nodal agency at the national level, has been actively involved in orientation and training of various personnel associated with the kala - azar control and during the experience of orientation, monitoring and supervision, a dire need for development of a comprehensive but short publication dealing with specific issues related to kala-azar control in India was felt.

This publication has been a humble initial attempt to make available a Hand Book for various categories of personnel connected with kala-azar control in India particularly at field level. Since this is the first attempt, we feel that there is a scope for improvement in

this publication which is proposed to be carried out in due course of time as an attempt of bringing out a more comprehensive publication. Suggestions from all readers are thus invited for its improvement.

The authors are thankful to Sh. P. P. Chauhan, Secretary (Health) and Dr. Narendra Bihari, Director General of Health Services Govt. of India, Ministry of Health and Family Welfare for providing constant support and encouragement in their endaevour to fight the menace of Visceral Leishmaniasis in India. Technical support provided by Dr. R.S. Sharma, former Director, NMEP is thankfully acknowledged. Sincere thanks are also acknowledged to World Health Organisation for providing support in bringing out this publication. We are highly indebted to all the experts, research workers and public health professionals working at various levels from whom we feel that the inspiration, information and knowledge has been gathered. Technical support by Sh. J. Sundara Rao. In reviewing manuscript is thankfully acknowledged. Assistance provided by S/Shri. S. Tudu, B. Soren, S.K. Chandna, Ram Ratan and Pyare Lal in preparing the manuscript is also acknowledged.

Authors

Delhi



LEISHMANIASES AND THEIR DISTRIBUTION

Leishmaniases are vector borne infections transmitted by female sandflies and caused by a protozoan parasite of the genus *Leishmania*. Different species belonging to this genus are responsible for morbidity due to different diseases in many endemic parts of the World. Leishmaniases have been recorded in over 80 countries in the World and in over sixty, they are present in epidemic form. Three main species of this genus cause different types of clinical manifestations in man. *Leishmania donovani* causes Visceral Leishmaniasis (Kala-azar), *L. tropica* causes Cutaneous *Leishmaniasis* (Oriental sore) and *L. braziliensis* causes the Mucocutaneous *Leishmaniasis* (espundia). In addition, *L. infantum* and *L. chagasi* also cause visceral leishmaniasis and *L. major*, cutaneous leishmaniasis in some parts of the world.

Though the clinical manifestations presented by different species are strikingly different, the parasites are morphologically indistinguishable. However, these parasites are biologically different. Further, the disease produced by one species is distinct from another ecologically. In this Chapter, a brief discussion follows for definition and distribution of different types of leishmaniases.

1. Visceral Leishmaniasis (VL) or Kala-azar

Visceral Leishmaniasis is a chronic disease caused by Leishmania donovani and characterized by an irregular undulating fever with often double rise in temperature, progressive cachexia, headache, malaise, progressive splenomegally, hepatomegally and emaciation, leucopenia and lymphadenopathy. It is a visceral infection of reticulo-endothelial system.

Kala-azar or Visceral Leishmaniasis is present in endemic form in India and China, Turkey and western and middle Asia, Sudan, Kenya, Somalia, Ethiopia, Morocco, Tunisia and Mediterranean islands and from Venezuela to northern Argentina in South America. The disease is more or less completely absent from the western Hemisphere except the eastern parts of Brazil.

In India, endemic belt extends from Meghalaya, Assam (Brahmaputra Valley), Bihar, Uttar Pradesh, West Bengal, Coastal Orissa, Coastal Andhra Pradesh and Tamil Nadu. Presently, disease is endemic only in Bihar and West Bengal with sporadic incidence in Uttar Pradesh. Sporadic cases in past had also been recorded in J & K, Gujarat, Punjab, Himachal Pradesh and Chandigarh.

2. Cutaneous Leishmaniasis (CL)

Cutaneous leishmaniasis (CL) commonly known as Oriental sore, is caused by *L. tropica* which, as already mentioned, is morphologically indistinguishable from *L. donovani*. This is a disease in which mainly the cells of reticulo-endothelial system of skin are infected and there is no involvement of visceral tissue. The disease is manifested by appearance of a cutaneous lesion which is commonly termed as Oriental sore or Delhi Boil. The lesion begins as a small nodule which ultimately ulcerates. Though the nodule may be similar to that of Post Kala-azar Dermal Leishmanoid (PKDL), it can easily be differentiated by the presence of ulceration and usually bigger in size than that of PKDL. However, widely varied clinical manifestations of CL are common. Besides *L. tropica*, *L. major* and *L. aethiopica* also cause cutaneous leishmaniasis in different parts of the world.

There are three major types of cutaneous leishmaniases:

(i) Anthroponotic or Urban Cuteneous Leishmaniasis (ACL)

It is caused by *L. tropica*. There is no zoonotic reservoir involved in transmission. The ulcers produced are dry and usually heal spontaneously in about a year.

(ii) Zoonotic or Rural Cutaneous Leishmaniasis (ZCL)

It is caused by *L. major* and a zoonotic reservoir is invariably involved in the transmission cycle. Lesions are usually multiple, inflamed and ulcerated. These are commonly complicated by secondary infection.

(iii) Diffuse Cutaneous Leishmaniasis (DCL)

It is caused by *L. aethiopica* and appear as multiple nodules or lesions on face and extremities.

Cutaneous Leishmaniasis is prevalent in central and western India, Iran to Central Asia, Central Africa along the shores of Mediterranean, Syria, Arab and Messopoteamia. In India, disease used to occur in west Rajasthan, Gujarat, western Uttar Pradesh and Punjab. *L. tropica* and *L. major* are the causal agents. Prevalence of both anthroponotic cutaneous leishmaniasis (ACL) in urban situations and zoonotic cutaneous leishmaniasis (ZCL) in rural situations have been recorded in India. In rural zoonotic foci, dogs, rats and desert gerbils are primarily involved in maintenance of parasite in zoonotic cycle.

3. Mucocutaneous Leishmaniasis or Espundia

The disease is caused by *L. brazilliensis* and *L. panamensis*, both prevalent in New World only. Though primary lesions appear on skin only, very soon the mucosal tissue is extensively involved and in almost all cases, nasal mucosa invariably manifests lesions.

In addition to these three major types, some other species cause leishmaniasis of local importance like *L. peruviana* causing Uta and, *L. maxicana*, *L. panamensis* causing cutaneous leishmaniasis, etc. These are not discussed in detail here.

Visceral Leishmaniasis in India

Visceral Leishmaniasis probably used to occur in India even in middle ages. However, earlier epidemics of visceral leishmaniasis were often labelled as of malaria due to similarity in signs and symptoms of both the diseases i.e. splenomegaly and fever. Bengal is one of the oldest known kala-azar endemic areas in the World. The earliest recorded outbreak of fever which could be ascribed to kala-azar was in 1824-25 in Jessore (Now in Bangladesh). The disease entity described as "endemic Cachexcea" of the tropical countries that are subjected to paludal exhalations by Twining (1835), in Calcutta was most likely 'Kala-azar'.

Prevalence of disease in South India could be recognised only in the beginning of 20th Century when Christopher Donovan discovered the parasite. With slow spread, disease became prevalent in almost all parts of the eastern half of the country right from north to south except north-west, due to climatic conditions which restricted transmission and extension of the disease to these parts.

Epidemiological situation in erstwhile endemic states WEST BENGAL

In India Kala-azar is known to exist since long and with the help of available records it can be traced back as far as 1824-25. The outbreak of fever in 1824-25 in Jessore which was known as 'Jwar-Vikar', can be ascribed to kala-azar. It is said that this epidemic caused death of no less than 7,50,000 people during a period of three years. After eight years in the year 1832-33, the disease appeared in Nadia district and in 1857 in Hoogly district. Burdwan fever of 1854-75 was also attributed to kala-azar (Roger). Epidemic fever of Dinajpur and Rangpur districts could be attributed to kala-azar.

The number of kala-azar cases reported by different hospitals and dispensaries of the districts of West Bengal during the period of 1931 to 1942 are given in the table. Kala-azar outbreak appeared in Calcutta after famine of 1943 and reached its peak in 1946. During the period between 1971-1977 only 11 (eleven) indigenous kala-azar cases were reported from West Bengal.

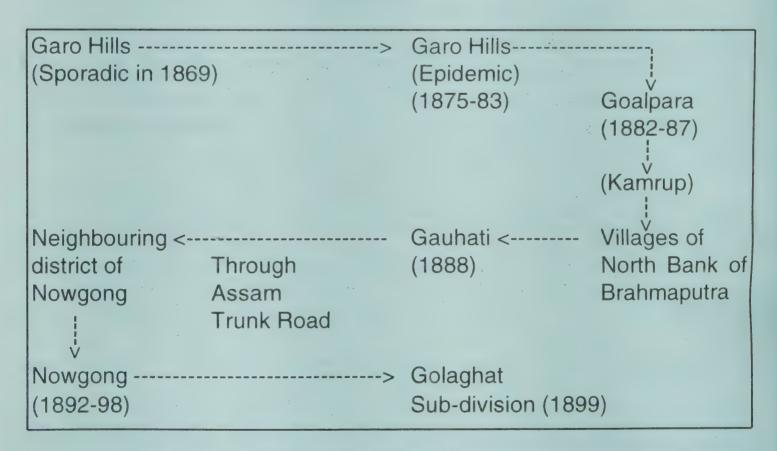
Year	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942
District												
Malda	5326	9075	8764	8140	9060	7034	3844	4393	4320	5771	4847	4592
Murshidabad	5737	9009	9175	7721	5472	5886	6532	5842	6038	5768	9392	
24-Parganas	9155	5873	7737	2587	2295	2009	1014	. 1491	1007	989	1071	539
Dinajpur*	2992	3715	6625	7228	5772	6073	5844	6606	6767	7166	6840	6890
Jalpaiguri	748	795	958	965	818	881	869	656	782	876	685	799
Darjeeling	356	653	951	1423	1308	2215	1648	1258	1050	915	987	869
Birbhum	54	63	74	94	98	67	68	95	97	70	88	86
Bankura	138	101	71	65	80	28	20	21	34	. 28	18	15
Modnapur	1258	1097	1025	770	441	447.	194	123	96	91	44	95
Hooghly	3249	2339	2460	2154	485	1308	1170	932	1074	949	1892	2957
Howrah	955	765	816	947	347	130	93	79	68	106	51	58
Nadia	6549	5415	5579	4794	3902	4150	3934	3974	3428	3810	3834	5114
Burdwan	3407	2373	2679	4187	2299	1832	1428	899	2361	1902	797	813

N.B. The data for Cooch Behar and Purulia districts are not available.

^{*} At the time of partition of the country a portion of the district has formed part of Bangladesh, the portion remaining in West Bengal is shown as West Dinajpur subsequently.

ASSAM

With the British conquest of Assam and improvement of communication, the disease spread to Assam which was then became popular as "Sarkari Bemari". as its spread was associated with progress of the development work of Assam Trunk Road Construction. This was in fact due to movement of labour aggregation and migration with construction of the highway. The first outbreak took place in 1869 in the Garo hills (Scott, 1942). Thereafter it descended in the valley where it developed in full scale epidemic between 1890-1900. The worst affected area was Nowgong district. Instead of having positive population growth, during the decade 1891-1900, this district was having population decline of 24.78 per cent. Movement of this epidemic was well defined and could be traced and demonstrated.



Large number of villages in Garo Hills district were abandoned, Deaths due to fever increased from 9000 to about 13000 per year in Goalpara and from 5000 to 7500 per year in Kamrup. As a result about 1/3 of the population lost their lives during 1892-98. Records of Assam areas showed that there were epidemics in the year 1885,

1897, 1913, 1925 and 1944 showing a mean inter - epidemic period of 15 years. It was also said that the epidemics were lasting over a period of five years or more and during the inter epidemic period, the number of cases used to drop down to about a quarter. Decadal number of fresh kala-azar cases reported from highly endemic district of Assam from 1920 to 1949 are given in below.

District		Total		
	1920-29	1930-39	1940-49	(1920-49)
1. Nowgong	71101	19540	33560	124201
2. Garo Hills	13223	9001	18705	40929
3. Goalpara	35856	15882	24969	76707
4. Darrang	26034	8084	21516	55634
5. Sibsagar	19319	16045	30296	65660
6. Kamrup	47150	16004	16005	79159
7. Cachar	3194	5020	11056	18270

BIHAR

The first appearance of kala-azar in Bihar probably occurred in 1882. "Kala-Dukh" in Purnea (Brown: 1898) was attributed to kala-azar. The notable epidemics came in 1891, 1917 and 1933, when the number of cases reached a colossal figure of 91,942. Thereafter, there was a declining trend towards 1939-40 possibly because of opening of special kala-azar treatment centres. No specific data is available for the period following 1940 but the records of Patna Medical College Hospital indicate fall in admission of kala-azar patients during the period 1942-1954 and no case, was admitted during the period from 1958-1970. The Directorate of Statistics and Evaluation Department of Bihar Government records show that from 1955 to 1960 there was definite decline in cases of kala-azar coming down to 3913 in 1960. From, 1961 there was a further decline, only 81 cases were admitted at Children's Hospital, Patna Medical College between 1961 and 1972 but 90 cases were

admitted during the period 1973 to 1978 in the same hospital. During 1975, 6 patients, 5 from Bihar and 1 from Madhya Pradesh (Bastar district) were treated at Calcutta National Medical College hospital. Since the movement history of the patient from Bastar was not traced, the focus of infection remains doubtful.

TAMIL NADU

There is no definite history of large scale epidemics of kalaazar in Tamil Nadu like Bengal, Assam and Bihar but cases were there which is evident from the discovery of L.D. bodies in Madras City by C. Donovan in 1903. In Tamil Nadu three areas, Madras city, Ramanathapuram and Tirunelveli were reporting kala-azar cases. The cases reported to Medical Institutions from three areas during the year 1945 to 1952 were 17932, 31581 and 2240 respectively. During the period from 1958 to 1961, cases were 1264, 4519 and 10329 respectively. Number of cases reported from Madras city during the years 1959 and 1960 were not available. During the year 1959 abnormally high number of cases i.e. 10137 were reported from Tirunelveli district, the reasons for that could not be explained.

Though kala-azar was endemic in these areas of erstwhile Madras State (Tamil Nadu), this southern focus was considered to be an independent and isolated focus and was never considered as an outcome of contiguous spread and extension of the endemic foci of eastern India.

Resurgence of Kala-azar in India

As a collateral benefit of DDT spray under NMEP, the incidence during 50's and 60's became negligible and people forgot about kala-azar. This decline went even upto such extent that the drug manufacturers either scaled down or totally stopped production of anti-kala-azar drugs.

Spraying of residual insecticide was withdrawn under NMEP in a phased manner from different areas from 1962 onwards on entry of malaria units into consolidation and maintenance phases. As the programme advanced, more and more areas entered into consolidation and maintenance phases and this resulted in slow build up of sandfly population, the vector of kala - azar. In seventies kala-azar cases started being reported from many institutions signalling a simmering outbreak. The hospital records of four districts of north Bihar viz. Muzaffarpur. Vaishali, Sitamarhi and Samastipur during 1974, 1975 and 1976 showed that, the total number of cases of kala-azar reported was 40, 850 and 1371 respectively. In 1977, a sample survey was conducted by the National Institute of Communicable Diseases, Delhi that showed an estimated number of 70,000 cases in the state of Bihar with 4500 deaths (Sanyal et al, 1979).

Post Resurgence situation of kala-azar in India.

After initial resurgence in four district of Bihar that became well perceptible by 1974, kala - azar started spreading to adjoining areas slowly and gradually. This spread was a rather silent spread as by that time it had not been recognized as a serious problem of public health consequences. However, by 1977, several districts of Bihar registered fresh kala - azar cases and the problem became wide spread. An attempt to control kala - azar in Bihar was made during the period 1977 to 1979 under the guidance of NICD. The incidence of kala-azar based on reported data for this period is indicated below:

Year	Cases	Deaths
1977	18589	229
1978	41980	62
1979	25472	28
1980	13620	25

Though the attempt resulted in a decline in reported incidence of kala-azar, the efforts were not continued and thus the transmission continued and ultimately the trends assumed serious proportions in eighties. The disease also got opportunity to spread even to West Bengal where indigenous transmission became perceptible in 1980s.

As is evident, this attempt of 3 years duration for 1977 to 1979 aimed at control of kala-azar in Bihar did not help in effectively controlling kala-azar and the spread continued unabated leading to widespread occurrence of kala-azar. The disease that resurged initially in 4 district of Bihar in 1974 slowly and gradually spread to adjoining areas and by the late eighties, became wide spread in entire north Bihar, a few pockets in south Bihar like Santhal Paraganas and several districts of West Bengal.

The distribution and prevalence of kala-azar changed dramatically in the wake of regular residual insecticidal spray instituted all over the malarious areas of the country under NMCP/NMEP for interruption of malaria transmission from 1953/1958-59 onwards. The DDT spray operations reduced the 'sand fly', (Kala-azar Vector) populations to very low levels resulting in interruption of 'Kala-azar' transmission, and in virtual elimination of the disease. But it has again taken serious dimensions since late eighties, certainly because of slow build up of vector population and resumption of disease transmission as a consequence of withdrawal of residual DDT spraying under NMEP from 'kala-azar' endemic areas as early as 1963-64.

After resurgence of VL in India in seventies, sporadic cases were recorded in and around Madras also till 1983 and since then no indigenous case has been recorded. This resurgence was also independent of eastern India.

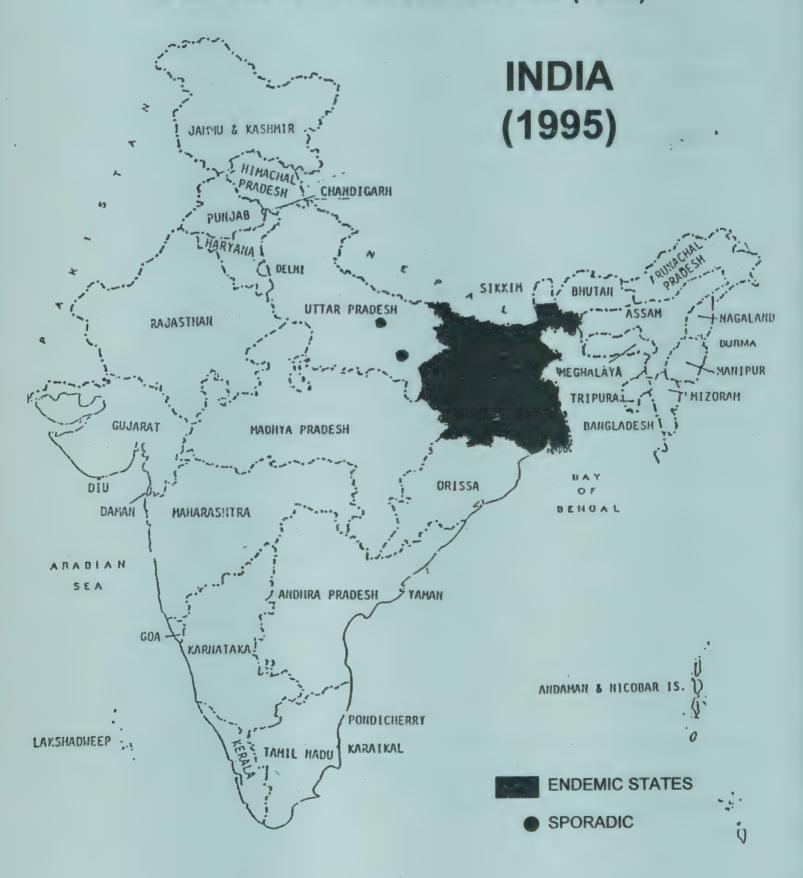
Besides the above mentioned endemic areas, kala-azar was also reported from some non-endemic areas in sporadic manner.

During 1967, 2 cases of confirmed kala-azar were reported from Gujarat for the first time.

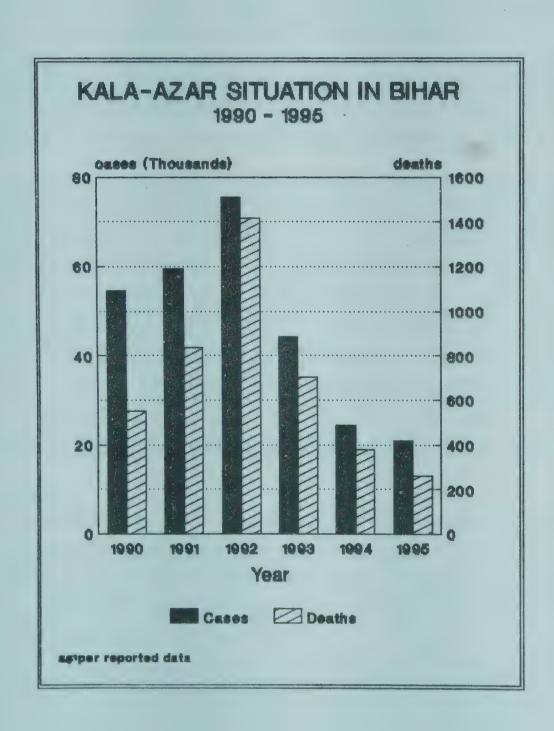
In Gujarat, Baroda Medical College reported 26 cases during the period 1966-76. Post Graduate Institute of Medical Education and Research, Chandigarh treated 24 patients of kala-azar during the period 1967-1977. Of these, 19 cases of kala-azar were from non-endemic areas. The distribution of cases were 8 from Himachal Pradesh, 3 from Jammu, 2 from Punjab, 3 from Haryana (two of which visited endemic zones in eastern sector), 2 from Uttar Pradesh and 1 from Chandigarh. Remaining 5 patients were from endemic areas of Nepal (4) and Bihar (1). However, 2 patients of Nepal were residing in Himachal Pradesh for over six years. Kala-azar was considered improbable in Kashmir before 1948-49 when 12 cases were reported from State Army and Militia. Of these, at least 5 cases were proved to be indigenous. During the year 1974 also, two cases were reported from Jammu. Two indigenous cases of kala-azar have also been reported from Himachal Pradesh in 1984.

Since 1977 the NMEP Directorate is collecting information regarding kala-azar incidence from different States and Union Territories. There is no regular surveillance machinery for detection of kala-azar cases; the cases which report at their own to hospitals, PHCs and Dispensaries are recorded. The cases which are treated by private practitioners for some reason or the other, are not included in the reports furnished by State/UTs. Thus it goes without saying that the data projected does not reflect the exact magnitude of the problem but definitely it shows the trend of the disease as well as its spread.

KALA-AZAR AFFECTED STATES (1995)



Kala-azar incidence recorded an increasing trend after resurgence till 1992 with sharp decline from the year 1993 onwards. The decline in VL morbidity and mortality during 1993 against 1992 was 41 and 50 per cent respectively. During 1994, a decline of 66.73 % in morbidity and 72.9 % in mortality had been recorded as against 1992 base year with highest morbidity and mortality. This could be attributed to launching of an intensified kala-azar control scheme in Bihar during 1991-92.



LEISHMANIA - THE PARASITE

Leishmania belongs to phylum - Protozoa which is typically characterized by the acellular body. The genus represents a group of most primitively reproducing protozoans of subphylum Sarcomastigophora (Flagellates).

The genus consists of two subgenera:

- i. Leishmania Sajjanova 1982.
- ii Viannia Lainson & Shaw 1987.

Leishmania (Leishmania) donovani donovani and L.(L). infantum are the causal agent of visceral leishmaniasis in the Old World. L.(L.) chagasi is the causative organism for VL in New World.

In Old World, cutaneous leishmaniasis is caused by *L.(L.) major*, *L.(L.) tropica* and *L.(L.) aethiopica*. A number of species including *L.(L.) mexicana* complex *(amazonensis; venezuelensis* and *mexicana)*, *L.(L.) garuhami* and *L.(L.) pifanoi* cause cutaneous leishmaniasis. The species of subgenus *Viannia* namely *L.(L.) brazilliensis* complex *(guvanensis, panamensis* and *peruviana)* also cause cuteneous leishmaniasis in New World.

Discovery of the parasite Leishmania donovani

The discovery of *Leishmania donovani* is an interesting story. Kala-azar has been endemic in India particularly in Bengal for a long time and was recognised as a distinct disease only in latter part of the nineteenth century. Because of close similarity in clinical manifestations of kala-azar and malaria, it was initially thought to be a severe form of malaria. Due to appearance of severe epidemics of disease like kala-azar around 1875 particularly in Assam besides Bengal, the efforts to describe the correct etiology gained momentum.

The first hypothesis was put forward by Giles in 1887 suggesting kala-azar as ancyclostomiasis due to presence of hookworm ova in every case he examined, though it was only circumstantial. The theory of malarial origin was reviewed by Rogers (1896) and Ross (1900). Manson (1903) put forward a hypothesis suggesting causative organism as trypanosome. The first record of correct etiology came from Leishmania in 1903 when he published his observations on a peculiar parasite found in an autopsy specimen of spleen of a soldier died in England in 1900 due to fever he contracted at Dum-Dum in Calcutta. A simultaneous report was published by C. Donovan (1903) from Madras describing the similar parasitic bodies in splenic aspirate of a kala-azar case. In the same year, Ross gave the name *Leishmania* to these parasitic bodies and since then the kala-azar parasite has been known as *Leishmania donovani*.

L. infantum was described by Cathoire (1904), Pianese (1905) and Nicolle (1908) in children suffering from "splenic anemia of infants" prevalent in most of the Mediterranean countries. However, L. infantum is now considered identical to L. donovani. Similarly, Aspland (1910) in China and Bousfield, Thomson and Marshall (1911) in Sudan also described kala-azar. Chagas et al (1937) discovered Leishmania bodies in viscerotome examination for yellow fever and gave the name Leishmania chagasi. However, this parasite was also identical to Leishmania donovani.

Morphology

Though different *Leishmania* species are morphologically identical, two morphologically distinct stages exist in the life cycle of all species of *Leishmania*, these are:

- (i) Amastigote (aflagellate) or Leishmania stage and
- (ii) Promastigote (flagellate) or Leptomonad stage.

(i) Amastigote or Leishmania stage

The Leishmanoid or aflagellate stage is found in man or other vertebrate hosts. This stage is represented by a minute oval body measuring about 2 to 6 micrometres in length and 1 to 3 micrometres in width. This stage is found intracellular particularly in the reticulo-endothelial system. The parasite prefers to stay in macrophages like monocytes, lymphocytes and the cells of bone-marrow, spleen and liver. A very high density of these parasites may be seen in spleen, liver and bone marrow. Detection of parasite in peripheral blood has been a rare phenomenon. The body structure of *Leishmania* can be examined only in a well stained preparation under microscope in high magnification. Following structures can be observed;

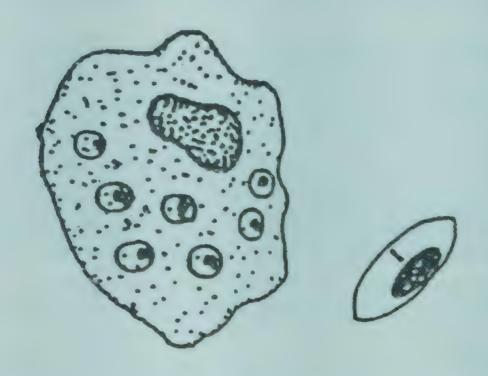


Fig. Amastigote

The ovoid body enclosed in a thin double cell membrane has a relatively large nucleus which is placed peripherally. Below the membrane, there is a row of 130 to 300 hollow fibrils. Though there is no flagellum, a conspicuous kinetoplast consisting of a rod like structure or parabasal body and a dot like basal body or blepheroplast can be seen at right angles to the nucleus. A delicate thread

connects the two parts of kinetoplast and an axoneme arises from the blepheroplast and extends up to the anterior tip. The axoneme represents the root of the flagellum of the leptomonad stage. The cytoplasm also contains a mitochondria and multiple neutral red vacuoles besides riboneucleic acid containing volutin and basophilic granules.

(ii) Promastigote or Leptomonad stage

This stage is found in the vectors as well as in the culture medium. Leishmania in this stage of life cycle is an elongated structure with a well developed single flagellum which is equal to or even longer than the elongated body of the parasite. The length of the Leptomonad varies from 15 to 20 micrometres and the width from 1.5 to 3.5 micrometres whereas the length of flagellum itself measures from 15 to 28 micrometres. In the gut of the infected sandflies (vectors), numerous flagellates can be seen. Leptomonad has a double cell wall below which there is a row of hollow fibrils. A large vacuole is characteristic of the flagellate. A conspicuous kinetoplast is located at the anterior end. The origin of a single, highly motile flagellum is very close to the anterior end of the kinetoplast. Since Leishmania or aflagellate stage undergoes transformation into Leptomonad or promastigote stage in the body of vector sandfly and culture medium before reproduction. Sometimes various short, round and broad forms can be seen which are developing stages of leptomonad. However, number of such form is always very small. These have been labelled as nectomonad.

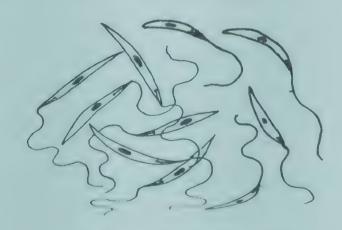


Fig. Promastigote

Life Cycles of Leishmania donovani

In the life cycle of L. donovani, though two morphologically different forms exist but there are no sexual (reproductive) and asexual (non-reproductive) stages. The reproduction of both the morphological forms is by binary fission. Leishmania or amastigote form is found in vertebrate host (man) and is commonly known as LD bodies (L. donovani Bodies). After reaching into the gut of sandfly fed on a kala-azar patient, the ovoid LD bodies grow into elongated flagellated structures to become Promastogotes. Reproduction inside the gut starts only after this transformation. The promostigotes undergo intensive multiplication by way of simple binary fission even with the blood clot inside the gut of vector sandfly. These slender, long, electron dense structure called "nectomonads" attach to the midgut by insertion of flagellum in the microvilli. While migrating towards anterior part of gut, nectomonads transform into stumpy nondividing electron light forms "hepatomonads", which attach to the cuticular lining through distally expanded form of flagellum bearing "hemidesmosomes". Subsequent forward movement of parasite brings about further morphological changes to transform into "Opistomastigotes" which finally transform into promastigotes by the time they reach buccal cavity. The whole process of morphological development of promastigotes has been described by Killick Kendric et al (1974). This process takes 6-9 days. The multiplication continues and the leptomonads aggregate in the form of rossette. Due to heavy density of leptomonads, the buccal cavity becomes blocked and the leptomonad get regurgitated when the infective sandfly feeds on a vertibrate host. In vertibrate host, parasites shed flagellum, transforms into ovoid bodies and invade the cells of reticuloendothelial system where they continue to multiply by binary fission. This ultimately leads to proliferation of these tissues particularly spleen, liver, bone marrow and lymph nodes.

When the LD bodies drawn from a human host are cultivated in a culture medium, they also get transformed into leptomonad form and only flagellates can be seen growing in the culture medium.

VISCRERAL LEISHMANIASIS - THE DISEASE

The reaction to parasization of the reticulo-endothelial cells in internal organs leads to a great increase in their number specially in the spleen and liver which become protesquely enlarged. The onset of the disease is often with the symptoms suggestive of malaria or typhoid. Kala-azar may actually be precipitated by these diseases. There are evidences that there is a high degree of natural resistance to kala-azar infection and probably the parasites are held under control in many latent infections in which no symptoms appear until resistance is lowered.

The disease exists in two forms:

(i) Kala-azar or Visceral stage

When an infected sandfly bites a healthy person, leptomonad from of parasite are inoculated into the body of human host. These parasites shed their flagella and transform into the Leishmania stage which infects the cells of macrophage system. The incubation period is variable from few weeks to even eighteen months or more depending on the nutritional and immune status of the individual. After onset, there is irregular fever with often double rise like P. falciparum malaria, enlargement of spleen and liver, rheumatic aches, anemia, progressive emaciation and lymphadenopathy. The leucocytes reduce in number (Leucopenia) and the skin often becomes edematous. Untreated patients usually die in a few weeks to a few years, usually due to some intermittent infection which the patient fails to fight with his already infected macrophage system. The major sites of L. donovani infection in human body include spleen, liver, bone marrow, lymph glands and lymphocytes and monocytes in blood.

(ii) Post Kala-azar Dermal Leishmaniasis (PKDL)

In some of the treated cases of kala-azar which recover from visceral infection or systemic disease, whitish spots develop in the skin which may grow into different shapes and sizes. This manifestation of *L. donovani* presence in human body is known as Post kala-azar Dermal Leishmaniasis (PKDL). As a result of. administration of anti kala-azar drugs to the patient of visceral leishmaniasis, some of the parasites escape the lethal effects of the drug and migrate to the skin tissue where the drug concentrations are extremely low. Immune response may also force parasite to leave viscera and localize in skin tissue. Here they lie dormant for considerable time before starting multiplication. This condition is also observed in kala-azar cases in which there has been a sponstaneous cure. These skin manifestations have high density of parasites. The manifestation of Post Kala-azar Dermal Leishmaniasis usually appear after one, two or several years of kala-azar disease. Per cent prevalence, however, may vary to a great extent. There are three types of PKDL manifestations:

(i) Erythematous patches

These appear as depigmented areas on the skin in a butterfly pattern (butterfly erythema) particularly on face. These are considered as early manifestations. These patches are photosensitive and enlarge as reaction to natural light.

(ii) Macular lesions or Depigmented macules

These appear as depigmented areas on the skin of trunk and extremities i.e. mainly on the abdomen and hands and feet. The face is usually not affected. These are also considered as early lesions. The depigmented macules often resemble patches of leperomatous or tuberculoid leprosy but the loss of pigment as well as sensation is not as complete as in leprosy.

(iii) Nodular lesion

These appear as yellowish pink soft nodules on the skin which never ulcerate. These are considered as the manifestations of later stages and usually replace the erythematous patches and depigmented macules. However, sometimes these may appear in the beginning itself.

Though in past, PKDL used to be considered as a sequelae of kala-azar disease, there are reports of dermal manifestation of *L.donovani* infection in patients with no past history of active visceral disease and is being labelled as dermal leishmaniasis. However, the probability of a mild kala-azar with subsequent spontaneous cure due to moderate to low parasitimea and better immune responses of the individual needs to be considered carefully in such cases as these manifestations are often more likely to be ignored by the patients or masked by some other concurrent disease. Further, a few workers have concluded about the dermal manifestation of *L. donovani* directly without patients passing through an acute kala-azar phase and this probability needs to be considered in PKDL cases.

The PKDL patients do not suffer from any clinical or pathological disease except for the cosmetic effects of the lesions which often continue unattended.

PATHOLOGY

The preferred sites for inhabitation of *L. donovani* in the body of human host are the tissue of reticulo-endothelial system particularly the macrophage system. The most heavily infected organs are the spleen, bone marrow, lymphatic system and monocytes of the peripheral blood. The reaction to parasitization of a particular organ is *hyperplasia* and as a result, an overall enlargement of the infested organ takes place. Pathological changes, as a reaction to parasitization by *L. donovani*, occur mainly in four organs namely spleen, bone marrow, liver and lymph glands.

Spleen

There is a marked enlargement in the size of spleen due to hyperplasia. There is perisplenitis making the capsule of the enlarged spleen thickened and the spleen becomes very soft. There is total absence of fibrosis and the splenic tissue is friable (even thumb pressure breaks the material).

The vascular spaces in spleen become dilated and are filled with excessive blood. A large number of *L. donovani* can be seen within the reticular cells of Bilbroth cords which are considerably increased. The malpighian corpuscles disappear completely due to the pressure of hyperplastic tissue. The plasma cells are also increased in most of the cases.

Bone Marrow

The hyperplasia in bone marrow results in leucopenia particularly neutropenia. The haemopoietic activities are disturbed. The leucopenia is directly related to the extent of hyperplasia and as such increases as the disease advances. Thrombocytopenia and

orthochromic normocytic anaemia appears. Most of the haemopoietic tissue is replaced by the proliferated and parasitized macrophage. There is also an increase in the number of plasma cells.

Liver

The hyperplasia in liver results in its enlargement which is noticed when disease becomes comparatively old. The parasites inhabit the Kupffer cells which increase in number as well as in size. The sinusoidal capillaries are dilated and filled with blood. Some of the liver cells show atrophy due to pressure of the hyperplastic tissue.

Lymph Nodes

Lymph nodes also get involved being the part of reticuloendothelial system. There is hyperplasia in these nodes also but the occurrence in Indian kala-azar is not universal, though it is a striking feature of kala-azar in China and Mediterranean where canines are the animal reservoir for *Leishmania*. In West Bengal also lymphadenopathy has been reported to be a usual feature.

In some cases in Bihar, lymphadenopathy has been demonstrated.

DIAGNOSIS

Diagnosis of kala-azar has always been a challenging proposition. In endemic areas, the disease presents typical clinical syndrome.

Clinical Features of Kala-azar

Kala-azar is a chronic and insidious disease. After the incubation period, which varies considerably in different patients, the onset of the disease is gradual. The patient gets feeling of general malaise and high fever for prolonged periods with often double rise in temperature. This follows with progressive spleen enlargement, anemia and loss of weight leading to progressive emaciation. As the disease progresses, hepatomegaly (liver enlargement) and lymphadenopathy (enlargement of lymph glands) particularly in epitrochlear glands become apparent. In classical Indian kala-azar, skin on face and feet becomes dark (hence the name kala-azar), hair tend to become brittle and may fall off.

Differential Diagnosis

A number of other common diseases present clinical picture like kala-azar making differential diagnosis utmost important. The diseases with similar clinical features include;

- (i) Chronic malaria
- (ii) Typhoid
- (iii) Tuberculosis
- (iv) Liver abscess
- (v) Brucellosis
- (vi) Portal Hypertension
- (vii) Histoplasmosis

In view of number of other diseases presenting clinical features more or less similar to kala-azar, confirmatory diagnosis of kala-azar is extremely important. Following laboratory examinations could be done for diagnosis of Kala-azar:

1. Hematological examination

Progressive leucopenia and severe anemia are striking features of *L. donovani* infection. There is a progressive decline in *total leucocyte count*. Differential leucocyte count gives a higher monocyte and lymphocyte count. The total erythrocyte and platelet count also decline but the decline in erythrocyte count is not comparable to the extent of leuocopenia. There is severe anemia and as such the hemoglobin contents of blood must be estimated.

2. Detection of presence of *Leishmania donovani* in the body

For determination of the presence of *L. donovani* in the body of the patient, a variety of tests are being used. The purpose of various tests is to determine the presence of *L. donovani* infection, the specific component detected by different tests is certainly different and therefore these tests have different epidemiological significance. Various tests employed may be summarized as below:

(a) Serological test

(i) Napier's Aldehyde test

The presence of *L. donovani* in the body initiates a parasitization response thereby resulting in increased protein contents in blood. The concentration of gamaglobulins in blood increases considerably. Formaldehyde has a tendency to bind these serum immunoglobulins.

The procedure is simple. Five cubic milimetre venous blood is drawn from the patient and left for clotting. Serum is separated by removing supernatent after centrifugation. To one ml of serum, 2-3 drops of commercial formalin (40% formaldehyde) are added. Opaque jellification like white of a boiled egg through which light can not pass, indicates a positive reaction.

Jellification time	Intensity of reaction
2 minutes	++++
20 minutes	+++
120 minutes	++
24 hours	+

The serum globulins increase in a variety of infections and thus this test is considered to be rather non-specific. A psotive reation may also be seen in diseases like Tubeculosis, Cirrhosis of liver, Malaria, etc. Further, in kala-azar also, the test becomes positive only when infection is atleast three months old and may remain positive even after six months of cure.

In view of these facts, the results of this test must be carefully interpreted by corroborating them with positive clinical findings and should never be considered in isolation.

(ii) Compliment Fixation test

The complement fixation test is to detect specific antibodies present in the serum. The test is essentially an antigen-antibody reaction in which the antibodies present in the serum are bound to an antigen. The antigen used in the test is prepared from either human tubercle bacilli (Witebsky, Klingenstein & Kuhn: W K K antigen) or from Kedrowsky's acid fast bacillus. The test is more sensitive as compared to aldehyde test as later becomes positive only after three months. However, cross reaction are observed in cases of pulmonary tuberculosis, leptomonas leprosy and *Mycobacterium* infections.

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(iii) Immuno-flourescent antibody test (IFAT)

Flourescent antibodies appear in the serum during the active phase of the disease and earlier than complement fixing antibodies during the prepatent period and persist for long after cure. The parasite antigen labelled with flourescent dye is conjugated with serum antibodies and seen under flourescent microscope.

(iv) Indirect hemagglutination test (IHA)

This is also based on the principle of antigen-antibody reation. The serum antibodies are conjugated with parasite antigens to observe agglutination.

(v) Enzyme Linked Immunosorbant Assay (ELISA)

Soluble antigen or *sonicated* extract of promostigotes is used to capture antibodies specific to *Leishmania*. Though sensitive and specific, it may give cross reactions with infections like malaria, tuberculosis, leprosy, etc. at very low titres.

(vi) Direct Agglutinition Test

The test is based on antigen-antibody reaction. Trypsin treated, stained and formalin preserved promastigotes are used as antigen which show agglutinition with specific antibodies present in patients serum. The test is performed at room temperature though the antigens are stored under controlled temperature in freezer. 50 microlitre (μ I) antigen suspension is added to 50 μ I diluted serum with thorough mixing for minimum 30 seconds in microtitre plate and incubated for 18 hours at room temperature (optimally 18 - 22°C). The results are read visually against white background and compared with positive (standard) and negative (blank) controls run parallel.

(b) Demonstration of Leishmania donovani

Detection of *L. donovani* (LD Bodies/Promastigotes) is the most specific test for diagnosis of kala-azar. Following methods are used for this purpose:

(i) Examination of spleen or bone marrow aspirates

Spleen and bone marrow are the sites prefered by *L. donovani* for inhabitation and thus parasites are available in extremely high densities in these organs. Since these tests are the most / specific and practicable, the techniques for spleen and bone marrow aspiration and staining of the smears are discussed here in detail:

(a) Splenic aspiration

Splenic aspiration is one of the most sensitive and widely used test and sensitivity may be as high as 95-97%

As a prerequisite for deciding splenic aspiration as the technique of choice for a particular patient, it is a must to determine prothrombin time and platelet count. If the prothrombin time is more than 5 seconds longer than the control sample and the total platelet count is less than 40000/cu. mm., the splenic aspiration for such patient is contraindicated. The procedure of splenic aspiration is given below:

Materials required

- 1. Glass microslides
- 2. Sterile Glass or Disposable syringe of 5 ml.
- 3. Sterile or Disposable needle or 21 or 23 gauge (1.25 inch long)
- 4. Sterile Tubes containing culture media.
- 5. Savlon, surgical spirit.

The patient should be examined thoroughly and spleen should be marked with a marker pencil after palpation. The patient should lie normally on the examination table and the procedure should be explained to him to avoid anxiety which may cause stress in abdominal muscle with consequent complications in otherwise normal splenic puncture. The abdominal skin should be cleaned first with savlon and then by surgical spirit. The needle must be attached securely to the syringe and then penetrate the skin of the abdomen midway between the edges of the spleen. The needle should then be oriented at an angle of 45 degrees to the abdominal wall. A suction pressure be created by pulling back the plunger in the syringe to about 1 ml. mark. Keeping the syringe at the same angle and maintaining the suction, the syringe should be pushed into the abdomen (spleen) to the full length of the needle and should be pulled back immediately. The suction should be maintained throughout the procedure. Caution must be taken to avoid sideway movement of the needle while piercing the spleen. Any attempt to change the angle of the needle midway may lead to rupture of the spleen. Further, the splenic aspiration should not be attempted if the spleen is not palpable atleast 2-3 cm. below the costal margin of the abdomen. In children the whole procedure should be completed as one single action.

The splenic aspirate so obtained should be transferred quickly as the aspirate tends to dry up in very short time. One of the best ways to fasten the procedure of transfer is to pull the plunger to the mark of 3 ml. to create a pressure inside the syringe and then pressing the plunger to push aspirate with pressure into the tube containing culture media. The remaining material is put on glass microslides and the smears may be prepared either with the help of needle itself or another microslide. Care should be taken not to crush or distort the cells in the aspirate. While using needle for smear preparation, it should be moved only in linear motion and not in circular motion. If a glass microslide is used, the margin should be touched on the slide containing aspirate in front of the aspirate

about 2-3 cm. ahead. The slide should be kept inclined at an angle of 45-60° and with slow backward movement, it should be brought in contact with the aspirate. With forward movement the aspirate is smeared on the slide. The smear should be made a little thinner than the thick film for malaria for best microscopic examination.

After splenic aspiration, the pulse and blood pressure should be monitored initially half hourly and after four hours, hourly for next six hours.

(b) Bone marrow aspiration

Bone marrow is another tissue of reticulo-endothelial system which is preferred by *L. donovani* to inhabit. A very high density of parasite is found in bone marrow. The preferred sites for bone marrow aspiration include sternum preferably manubrium of the sternum, iliac crest (posterior susperior iliac spine) and sometimes tibia.

Materials Required

- 1. Sternal puncture needle
- 2. Sterile glass or Disposable syringe 2 ml. with 21 or 23 gauge needle and 5 or 10 ml. syringe.
- 3. Sterile tubes with culture media
- 4. Glass microslides
- 5. Xylocaine 2%
- 6. Savlon, surgical spirit.

The sternal puncture needle or bone marrow aspiration needle is a special type of needle made of very strong steel. The needle is very stout and short provided with a well-fitting stylette. The needle is provided with an adjustable guard as a protection against excessive penetration across the bone.

The usual site for bone puncture is manubrium or the first or second pieces of the body of the sternum. The patient should lie on his back. The site of puncture should be cleaned and if hairy, should be shaved. The site should then be thoroughly cleaned with savlon and surgical spirit. Local anaesthetic like xylocaine 2% should be applied with the help of a 2 ml. syringe. Xylocaine must be dropped at 4-5 places and the skin should be peirced carefully to ensure that it penetrates the skin, subcutaneous tissue and periosteum overlying the site selected for aspiration.

After proper administration of local anaesthetic, the skin and subcutaneous tissue is punctured with a sterile sternal puncture needle. When the tip of the needle reaches the periosteum, the guard of the needle should be adjusted in such a way to allow only about 5 mm further insertion. The needle is then pushed farther in the cavity of the bone with a boring movement. A sudden reduction in the counter pressure ensures the penetration into the bone cavity. The stylette should then be removed by holding the needle in position and a 2 ml. or 5 ml. syringe should be fitted to the needle. About 0.3 to 0.5 cc bone marrow should be aspirated by pulling the plunger of the syringe. If bone marrow does not come in the syringe, the needle may be moved a little farther in the cavity of the bone and then the suction is applied.

The other site preferred for bone marrow is the posterior superior iliac spine or iliac crest. The point usually selected for the penetration of needle is just posterior to the anterior superior iliac spine or 2 cm posterior and 2 cm inferior to the anterior superior iliac spine. The needle should be inserted perpendicular into the iliac cavity. The posterior iliac spine covers a large marrow containing areas.

In very young children, the preferred site for bone marrow aspiration is the medial aspect of the upper end of the tiblia just below the level of tibial tubercle. In older children, the preferred site

is the posterior iliac crest as the tibia contains very little active marrow and the sternal puncture is not totally free from risk.

Preparation of Bone Marrow film

The preparation of bone marrow film is a careful procedure. In case of successful splenic aspiration, the aspirate is largely free of blood but bone marrow aspirates invariably contains substantial quantities of blood. This excessive blood interferes in the clarity of the slide for examination of LD bodies. Immediately after drawing aspirate in the syringe attached to the sternal puncture needle, a drop is put on clean glass microslides about one cm. from the edge. The excess blood should then be removed from each drop by sucking off with the help of fine Pasteur pipette / filter paper. A thin film of marrow fragments should then be made with the help of the edge of another microslide using it as spreader. The marrow fragments should be dragged behind the spreader edge to make a trail of marrow cells and to avoid crushing or distortion of the cells as has been explained under splenic aspiration procedure.

Lymph Node Aspiration

Though in some other countries like China, lymph node as piration/biopsy is the most preferred method of parasite demonstration, in Indian kala-azar the method has limited application as involvement of lymphatics with heavy parasitization in all VL cases is yet to be confirmed. However, it may be of particular use in cases of hypopigmented and macular PKDL where parasites are not readily demonstrated in skin smears.

Staining and examination of films of splenic / bone marrow aspirates

Every glass microslide on which a film has been prepared, should be labelled properly. The most common practice is to write the Patient's name and date or a reference number on the slide itself

with a marker pencil. A paper label may be affixed later. The marking with pencil is not spoiled during staining procedures. A prerequisite to successful staining is that the film should be properly fixed with Fixatives and should not be left dried for more than a few hours if it is not stained immediately. For best results, films should be stained as far as possible, immediately after it is air dried. The most popularly used fixative is methanol (methyl alcohol). The air dried slide should be dipped in a staining jar containing methanol for about 20 minutes.

Preparation of Stains

Although most of the stains used commonly are available commercially in ready to use formulation or as standards only to be diluted as per instructions, but the stains can also be prepared in laboratory. Procedure for preparation of two most commonly used stains i.e. Geimsa and Leishman, both based on Romanowsky dyes, are given below:

Geimsa Stain

Geimsa stain is available in powdered form. Weigh 1.5 gm of stain powder (Azure B type) and put it in a conical flask. Measure 100 ml of glycerol in a measuring cylinder and add to the stain poweder in the conical flask. Mix them thoroughly by using beads for about 90 to 120 minutes. Measure 100 ml. of methanol in a measuring cylinder and add to the conical flask containing mixture of Geisma powder and glycerol with constant stirring to ensure thorough mixing. Keep it for 7 days at room temperature. Filter the stain solution after 7 days through a good quality filter paper preferably Whatman No.1 Diluted stain should be prepared the same day when it is used.

Leishman Stain

Leishman stain is also available in the powdered form. Weigh

0.4 gm of stain powder and put in a conical flask. Measure 100 ml of methanol (acctone free) in a measuring cylinder and add into the flask containing the stain powder. Mix thoroughly and heat at 50° C for about 15 minutes. Stir or shake the mixture while heating for thorough mixing and uniform heating. Filter the solution and keep it at room temperature. The stain can be used immediately after its preparation but if allowed to stand, its quality is improved.

Buffer water used for washing of stained slides may be prepared either by using ready to use buffer salts available commercially or by the following method:

To prepare 0.666 M stock solution of phosphate buffer, solution A is prepared by mixing 9.1 gm KH₂PO₄ in 1 litre of distilled water. Solution B is prepared by mixing 9.5 gm Na₂HPO₄ or 11.9 gm Na₂HPO₄.2HO in 1 litre of distilled water. The two solutions are added till the desired pH is obtained.

Staining of Smears

The air dried and properly fixed slides are transferred to a jar containing Geimsa stain freshly diluted with 15-20 vol. of buffered water. The slides then should be dried at room temperature by keeping them upright for excess stain to drain out. If Leishman stain is used, the slide is first flooded with the stain solution by keeping the slide horizontal on a slide rack or on a tray with the help of two glass rods using them as support. After about 30 seconds to 1 minute, double the volume of water should be added and the slide should be left for staining for another 5-7 minutes. The slide should be washed in a stream of buffered water until it acquires a pinkish tinge. The slide should then be left upright for drying at room temperature.

Sometimes the slides are overstained, particularly when Geisma stain is used. Such slides may be dipped in phosphate buffer saline for a few minutes. The phosphate buffer saline may be prepared by mixing buffer solution with a solution of 9 gm NaCl in 1 litre of distilled

water in equal volumes. The phosphate buffer saline can also be used for diluting the stain standard instead of water in the first instance itself.

Examination of smears for Parasites

The well prepared and stained films should be examined under the microscope. Focus the slide under low magnification. Apply a drop of emersion oil on the film and adjust below the oil emersion lens of the objective. With slow movement of fine focusing rim, focus the film and observe the presence of *L. donovani* amastigote stage (LD Bodies) by recognising the following structures:

- an oval (occassionally round) body measuring about 2-4 microns along the longitudinal axis and found intracellularly.
- in the middle of the cell or more often along the side of the cell wall, a large nucleus measuring about 1 micron in diameter.
- a kinetoplast lying at right angles to the nucleus. Sometimes kinetoplast is may be found lying tangentially to the nucleus.
- an axoneme or rhizoplast representing the root of the flagellum originates from near the kinetoplast and extends upto cell wall
- a large vacuole alongside the axoneme, as a clear unstained space.

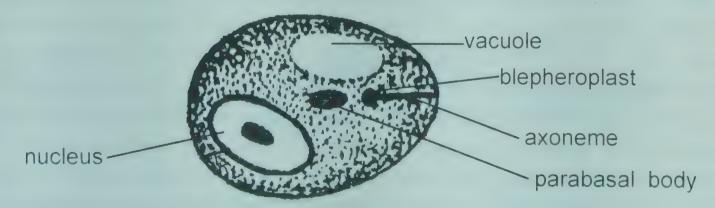


Fig. L.D. Bodies

The grading of parasitimea is done by the criteria suggested by the WHO (10x eyepiece and 100x oil emersion lens):

Grade	Average parasite density
6+	>100 parasites/field
5+	10-100 parasites/field
4+	1-10 parasites/field
3+	1-10 parasites/10 fields
2+	1-10 parasites/100 fields
1+	1-10 parasites/1000 fields
0	0 parasites/1000 fields
(Source: WHO TRS 793)	

It may be noted carefully that as the parasitimea decreases, it is mandatory to examine slide for longer duration and for larger smear area (No. of fields)

(c) Cultivation of Parasite

Cultivation of parasite is of great significance particularly in cases where the parasitimea is of low degree and where the presence of LD bodies is not confirmed as often happens during early stages of the disease. The culture can be developed from blood, bone marrow or splenic pulp. A variety of culture media are used. The most common is Novy, McNeill and Nicolle, commonly known as N.N.N. media. It consists of two parts of Salt agar and one part of defibrinated rabbit's blood. Salt agar is prepared by dissolving 14 gm of agar and 6 gm of sodium chloride (NaCl; common salt) in 900 ml. distilled water by heating and mixing in a water bath. When the agar is totally dissolved, the rabbit's blood is added while coolling and mixing it thoroughly by vigorous rotations of the tube. The media is then left for solidification at low temperature by keeping the tube in a slanting position. The tubes are stored below 4°c in freezer chest or a refrigerator. The material for culture i.e. blood, marrow or the splenic pulp, as the case may be, is inoculated in the water of condensation of N.N.N. media and

incubated at 32°C for a period of atleast one week and upto 3-4 weeks. In case of fine needle spleen biopsy, the needle itself is dipped in the water of condensation in culture media tubes. After one week, a drop of the water of condensation is taken out from the media tube and examined under microscope immediately for presence of flagellates i.e. leptomonad or promastigote form of *L. donovani*. These promastigotes can be seen as actively moving flagellates and if found, a permanent mount may be prepared after fixing and staining the slide as per standard procedures. The whole procedure should be performed under sterile conditions to avoid contamination. It is always helpful to run atleast 4-5 tubes for single sample to avoid contamination particularly while examining the contents for the presence of promastigotes.

A variety of other culture media like Tobie's Evans modified Tobie's. Difco Blood agar base (USAMRU), Locke's Hank's R.P.M.I. Schneider's, Mitsuhashi-maramorosch's, and Grace's insect media are used. Different media vary in their composition but the basis of media is almost same in all. In view of different composition, particularly in type of blood used, the rate of growth of promastigotes vary considerably. Among various modified media, Schneider's and Grace's media are considered better for culture but these are not available indigenously in India. Though N.N.N. media gives a slow growth of promastigotes, it is the cheapest media available.

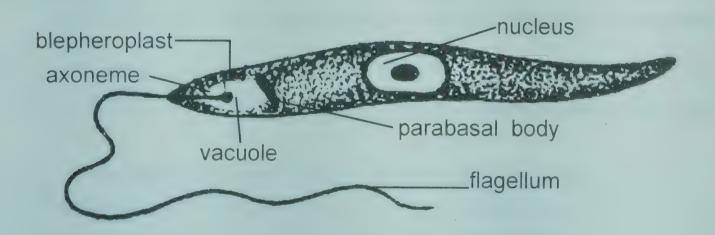


Fig. Leptomonad / Promastigote

Diagnosis of Post Kala-azar Dermal Leishmaniasis

The parasite demonstration in the dermal lesions is the diagnostic criteria for PKDL. The skin biopsy material can be collected with the help of sterilized needle from the nodular and erythematous areas. A homogenous smear is prepared on a clean slide and well stained preparations are examined under the microscope for the presence of amastigotes or leishmania stages (LD Bodies). Smears from depigmented macules usually do not show any amastigotes. Sero-diagnosis and culture preparations help in confirmatory diagnosis of macular lesions of PKDL.

In India, the diagnostic criteria followed under the Kala-azar control scheme are based on the recommendations of an Expert Committee (Dec 1995) that suggested criteria to be followed at various levels of health delivery system and the same are summarized below:

I. Rural/PHC

Clinical suspects

Persons with fever for more than 3 weeks of history, not responding to anti-malarial and antibiotics are to be suspected for visceral leishmaniasis. These suspected cases are to be referred to Medical Officer attached to the PHCs/BPHCs.

Use of Diagnostic Technologies

1. Napier's Aldhyde test - The Committee feels that this is to be continued in its present form until it can be replaced by other suitable tools. However, the Medical Officers must be made fully conversant with proper reading and interpretation of the result.

2. Wherever possible attempts are to be made to make available facilities for bone marrow aspirations, skin scraping and collection and despatch of blood for serological tests in difficult cases.

II. Secondary Level (Sub-Divisional and District Level)

- 1. As far as possible multiple diagnostic tools must be made available.
- 2. Emphasis must be given more on parasitological diagnosis which can be supplemented by serological tools like DAT, ELISA or any other tests.
- 3. Appropriate training and motivation of the health workers including Medical Officers at the secondary level must be undertaken to make the use of the diagnostic tools more meaningful.
- 4. Considering the bleeding diathesis in Indian Kala-azar, splenic aspiration should be reserved for Divisional/Institutional/Medical Colleges level. It should be done wherever needed by a person fully acquainted with the technology and only after excluding coaggulation abnormalities (bleeding time/prothrombin time) and if present, in such cases only after injection of Vitamin K once a day for 3 consecutive days.

III. Tertiary Level (Medical Colleges and Research Institutions/State level Hospitals)

- 1. This should act as the highest level of health care delivery system vis-a-vis kala-azar diagnosis and treatment. Therefore, other available diagnostic tools must be made available.
- 2. As far as possible parasitological diagnosis should serve as the basis for putting a patient to anti-leishmanial therapy. However,

other tools like serology may also be considered to supplement particularly the clinically positive but parasitologically negative cases after the field trial results are available.

- 3. Facilities for parasite isolation are to be made available particularly in view of assessment of cure following the chemotherapy. Culture should also be done in cases of Hypopigmented patch of PKDL which are usually smear negative.
- 4. Whenever needed in any such case, lymph node biopsy and culture should be encouraged.

IV. DIAGNOSTIC CRITERIA TO BE FOLLOWED AT VARIOUS LEVELS OF PROGRAMME IMPLEMENTATION

1. Primary Level

Any person with a history of fever for more than 3 weeks not responding to antibiotics, malaria being excluded, having palpable spleen and positive Aldehyde test may be subjected to anti-leishmanial treatment. In Aldehyde negative cases either the patient's blood or himself should be referred to higher centre.

2. Secondary and Tertiary level

A suspected case of leishmaniasis with parasites positive should serve as criteria for initiation of treatment. In cases where diagnosis by demonstration of parasite is not confirmed, Immuno diagnosis may be undertaken.

TREATMENT OF VISCERAL LEISHMANIASIS

Anti-leishmanial drugs

(i) Urea Stibamine

Sir U.N. Brahmachari developed an antimonial preparation in 1922 that ultimately became sheet anchor in treatment of Indian kala-azar. The drug Urea Stibamine (Stiburea), a compound of urea and stibamine (Sodium para-aminophenylstibinate) was brown amorphous powder that was injected intravenously after making solution with re-distilled water at room temperature. The adult dose was 0.05 gm initially, increased by 0.05 gm upto a dose of 0.2 gm (maximum dose), twice or thrice weekly upto 10-15 injections. The drug was highly effective with almost no significant unresponsiveness.

Due to virtual disappearance of kala-azar in sixties as a collateral benefit of malaria control/eradication activities, the manufacturing of this drug was stopped and now the drug is not available.

(ii) Sodium Stibogluconate

It is the drug of choice for treatment of Visceral Leishmaniasis. This drug is a preparation of pentavalent antimony and is usually available as 100 mg Sb⁺⁵ per ml of preparation. The drug is relatively less toxic and is quickly eliminated. The drug is administered intramuscularly and the maximum daily intake should not exceed 850 mg Sb⁺⁵. The drug is manufactured in India.

(iii) Pentamidine Isethionate

This is an aromatic diamidine. The drug is manufactured as white amorphous powder and is available in 200 mg ampules. The drug is dissolved in redistilled water just before use and should not be stored for more than 24 hours after reconstitution even under refrigeration. It is administered both intra muscularly (10% solution) and intra venously (1% solution). This drug is primarily used for treatment of cases unresponsive to antimonials, as it is relatively toxic and may even induce diabetes.

(iv) Amphotericin - B

It is a well known antifungal drug generally used for treatment of systemic fungal infection. This drug is effective against *Leishmania* donovani also.

Indiscriminate use of any drug leads to development of resistance among parasite / pathogens. It is important to rationalize use of various drugs to prevent / minimize the risk of development and spread of such a phenomenon. This rationalisation is of utmost importance in context of kala-azar because at present only 3 potent anti leishmanial drugs are available and unresponsiveness to drug of choice "sodium stibogluconate" is on increase. Appearance of unreponsiveness to pentamidine isethionate is also reported in a few cases.

The use of various antileishmanial drugs, dosage regmen etc. being followed under kala-azar control scheme in India are based on the recommendations of an Expert Committee (December 1995) and the same are summarized below:

CHEMOTHERAPY OF KALA-AZAR AT VARIOUS LEVELS OF PROGRAMME IMPLEMENTATION

A. Visceral Leishmaniasis:

I. First Line of Treatment

(i) Once diagnosed as kala-azar and a patient receiving sodium stibo gluconate outside without response:

Sodium Stibogluconate - 20 mg/kg body weight (maximum 850 mg/day) by single injection.

Route: Intra-mascular (IM) at peripheral level i.e. below district level and IM or Intra-veinous (IV) at district level and above.

Duration: 20 days, if partial response to 20 days treatment, then continue upto 30 days.

Check for parasite load in splenic or Bone Marrow smear at 20 days or 30 days as the case may be.

Criteria for Cure: Absence of L.D. bodies in aspirated material.

Contra-indication: Severe kidney, liver and heart disease.

Precautions: Make drugs like Adrenaline, Hydrocortisone hemisuccinate and other resuscitative measures available to guard against hypersensitivity reactions, etc.

Pregnancy: SSG should be given considering the danger to the mother if not treated as above.

(ii) SSG nonresponsive: No response to supervised SSG in 20 days and in case of partial response in 30 days and/or two courses of SSG in fresh cases, start second line of treatment.

(iii) Post Kala-Azar Dermal Leishmanoid:

4 to 6 course of SSG each comprising of 20 days as per the response with 10 days interval in the courses.

II. Second Line of Treatment

i) SSG nonresponsive cases as defined above.

Pentamidine Isethionate: 3 mg/kg body weight for children less than 12 years and 4 mg/kg body weight for children above 12 years and for adults.

Routes: Intramascular.

Duration: 15 injections on alternate days. Check for parasite in splenic or in Bone Marrow smear 10 days after the end of 15th injection.

Repeat the same as above if L.D. bodies found in the aspirated material.

Criteria for Cure: Clinical cure and absence of L.D. bodies in aspirated material three weeks and 12 weeks after completion of treatment.

Contra-indications: Diabetes mellitus, severe liver, kidney and heart disease.

Precautions: Give glucose drinks prior to injections. Make emergency drugs as noted under SSG available to guard against hypersensitivity reactions etc.

ii) Pentamidine nonresponsive:

No response to supervised 2 courses of Pentamidine.

III. Third Line Of Treatment

Pentamidine non-responsive cases.

Amphotericin- B:

- 0.5 mg first day.
- 0.5 mg second day.
- 5 mg third day,

and 0.5 to 1 mg/kg body weight fourth day onwards till cumulative dose of 1 to 3 gram in case of adults and 10 to 20 mg/kg body weight in case of children is reached.

Routes: Through Intra-veinous infusion in 5 per cent dextrose after mixing the drug in water for injection, very slowly in 6 to 8 hours.

Criteria for Cure: Absence of L.D bodies in aspirated material after 6 weeks and 6 months of the last dose.

Contra-indication: Kidney disease, severe liver and heart diseases.

Precautions: Stop the drug when signs of renal failure and those of hypokalaemia etc. appear. Make available emergency drugs as in SSG to guard against hypersensitivity reactions etc.

VECTORS

Leishmaniases are the vector borne diseases which exist either as zoonoses (in most of the endemic areas of the world) or anthroponosis (Indian sub-continent i.e. India, Bangladesh, Nepal). The vectors of various leishmaniases World over belong to Order Diptera of class Insecta (Phylum Arthropoda) that are characteristically recognised for one pair of functional wings and rudimentary clubbed shaped second pair called "Halteres" - the organs for maintaining equilibrium. The vectors of leishmaniases belong to group of insects commonly called as sandflies.

Sandflies are grouped in two sub-families namely Psychodinae and Phlebotominae. Only the members belonging to family Phlebotominae are transmitting agents for different types of leishmaniases.

The sandflies are usually associated with warm climate. About 500 or more species are distributed all over the tropics and sub tropics particularly in those parts of the world having atleast one month temperature of 20°C. Only very few species are considered to be the vectors and even fewer have been incriminated as vectors.

Sandflies specifically resemble with other Psychodid and with few other flies, but could be identified easily by their heavily clothed body with scales and hairs and their specific stance. It is important to remember that the identification of the species of sandflies solely depends upon the close examination of mounted specimen particularly the head and genitalia which bear the characters of taxonomic importance.

In India, so far three sandflies species have been incriminated as vectors of leishmaniases, they are:

- 1. Phlebotomus argentipes as only known vector of Visceral Leishmaniasis,
- 2. Phlebotomus papatasi as the vector of anthroponotic or urban cutaneous leishmaniasis.
- 3. Phlebotomus salehi as the vector of rural (zoonotic) cutaneous leishmaniasis.

The adult sandfly is a small, fuzzy, delicately proportioned fly, usually 1/4th of the size of the mosquito. The length of sandfly body ranges from 1.5 to 3.5 mm. The males and unfed females can pass through mosquito net easily. The elongated wings are hairy, held errect on the abdomen and are bigger than the size of the body. The body, wings and legs are heavily clothed with long hairs. The sandfly could be spotted easily because of the posture of the wings which are always held vertically erect when at rest.

The sub family Phlebotominae of family Psychodidae comprises of six genera namely *Phlebotomus*, *Sergentomyia*, *Brumptomyia*, *Lutzomyia*, *Warileya* and *Parvideus*. Of these, only two genera namely *Phlebotomus* and *Sergentomyia* are found in Indian subcontinent and are represented by about 46 species.

Biology and Bionomics

The sandflies are associated with warm climate and could be grouped into two categories namely species associated with wet zone and the species associated with arid zone and this association further delimits the distribution of different types of leishmaniases. Napier (1926) suggested ecological factors favourable for transmission of Visceral Leishmaniasis or kala-azar as:

- Alluvial soil
- High sub-soil water
- Monthly mean maximum temperature below 37°C
- Monthly mean minimum temperature above 7.2°C
- Annual rainfall 1250 mm or more

- Mean Annual Relative Humidity of 70% or more with more than 80% for atleast 3 months.
- Abundant vegetation
- Altitude below 600 metre

These factors inter-alia favour *Phlebotomus argentipes*, the only known vector of visceral leishmaniasis in India, to survive with high prevalence through greater part of the year facilitating transmission.

1. Adults

Distribution

P. argentipes is one of the widely distributed sandfly and is essentially the species of wet zone. It is widely distributed along side of the equator and in India, its distribution could be seen on the eastern half of the country and western limits could be marked by joining a line from Bombay to Delhi. Though commonly the sandflies are not found at the altitude above 600 metres, sporadic occurrence in India has been recorded in Kasauli at a height of 1200 metres and at 1300 metres in Pauri Garhwal in Himalayas. The species is predominantly distributed all along the eastern coast from West Bengal to Kanyakumari.

Seasonal Prevalence

As has already been mentioned, high relative humidity, warm temperature, high sub-soil water and abundance of vegetation favours proliferation of *P. argentipes* and accordingly, depending on this condition seasonal prevalence vary from area to area. In India the species is found through out the year in majority of areas of prevalence with complete absence in winter months in areas with extremes of temperatures like Assam. With the onset of warm weather coupled with humidity, the density increases till June with sudden decline due to high temperature. It is again followed by

increasing trend reaching the maximum during and just after the monsoon rain. A similar trend has been reported in Bihar with a minor peak in March/April and a major peak in August/September in densities of *Ph. argentipes*.

Feeding Behaviour

All species of sandflies feed on plant sugar and the females often feed on vertebrate host including man. The females of genus Phlebotomus feed on mammals whereas the Sergentomyia feed on reptiles. Recently Sergentomyia has been reported to feed on human host in isolated studies. Though P. argentipes are commonly known as zoophilic, it has been observed by various workers that the anthropophilic index largely depends on the sampling. For example, the samples collected from human habitation showed 69.6% anthropophilic index whereas the samples collected from cowshed in the same area showed only 21.6% anthropophilly. The same was true in the case of bovid blood index which was 96% in the cowshed and 44% in the human dwellings. This indicates clearly that P. argentipes are primarily indiscriminate (opportunistic) feeder and the type of bloodmeal largely depends on availability of host in its immediate vicinity. However, studies undertaken in Nilgiri hills indicate a high level of zoophilly to the extent of even 100%.

Flight Range

Though named as sandflies, these insects are not capable of flying very long distances. Their usual mode of flight pattern is a series of short erratic hopping in which the fly usually covers a distance less than 1/2 metre. Further, the dependence of the most of the vectors on humidity for their survival limits their movements. However, this distance may be even less or more depending upon climatic factors. The maximum distance from where a lebelled specimen. of *P. orientalis* could be recovered in Sudan, was 240 metres. However, most of the specimen were recovered from a

distance of 45 to 300 metres. In some earlier studies with mark/release/recapture technique, 92% of marked sandflies remained with 12 metres of the point of release and only 1% moved farther than 25 metres.

Resting Sites

The most favoured resting sites for sandflies include soil cracks and crevices, burrows (rodent burrows), tree holes, termite hills, caves, bird tunnel, in earthen mounds, under stone and foliage, etc. In eastern parts of India like Bihar & W.Bengal, *P.argentipes* prefers to rest indoors, about 8-10 times higher in cattle dwellings than the human dwellings. In western India, sandflies rest outdoors also in considerable numbers. As could be seen, all these resting places provide them dark and damp shelter where the microclimatic humidity is very high. They usually leave these shelters at dusk and are active in open in the evening and night. Usually sandflies remain active throughout the night but they are sensitive to decreasing temperature and air currents. Even a gentle breeze of 1.5 - 2 metre/second may greatly reduce their activity.



Fig. Sandfly

Longevity

Very limited studies have been conducted on the longevity of sandflies. In India *P. argentipes* have been reported to undergo 5 gonotropic cycles under laboratory conditions, duration of each cycle being 4 to 5 days at 26±2°C i.e. a minimum longevity upto 23-27 days under laboratory conditions. Field studies conducted on parity have also shown presence of triparous and "four parous" females in nature indicating probability of longevity under field conditions to be upto 16-20 days in a proportion of natural population. However, longevity in field is directly dependent on ecological factors.

2. Immature Stages

1. Egg

The freshly laid eggs are creamy white in colour which later become dark. The eggs are usually deposited in cracks and crevices with high organic content, humidity and darkness. Sometimes eggs are also found in loose soil. The eggs are glued to the surface through flattened while the convex side faces upwards. The egg shell has sculptors and their size varies from 0.336 - 0.432 mm x 0.096 - 0.160 mm. A wide range has been observed for total number of eggs laid per female (5-68). The eggs hatch in 3-4 days at 26±2°C in laboratory.



Fig. Egg

2. Larva

The creamy white larva with distinct head, thorax and abdomen have numerous hairs on its body. The larva feeds on organic matter available in the soil. There are four larval stages:

I instar

The delicate larva is whitish with a brown head capsule lacking eyes. A pair of black caudal bristles and presence of egg breaker on the posterior portion of head are the characteristic features of I instar. The average life is 2-4 days.

II instar

Presence of 2 pairs of caudal bristles, round 3rd antennal segment and absence of egg breaker are the features of II instar which lives for about 2-5 days.

III instar

Presence of 2 pairs of caudal bristles on completely dark last abdominal segment, partially elongated 3rd antennal segment and yellowish body hairs on a well developed larva help in identifying III instar which lasts for about 3-4 days.

IV instar

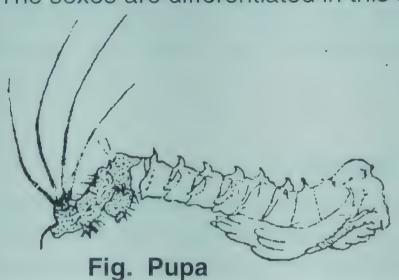
It is a well developed brown larva with dark brown head capsule, elongated, oval 3rd antennal segment with a pointed seta. Two pairs of spiracles and two pairs of well developed caudal bristles are conspicuous. The stage lasts for 4-7 days and transforms into pupa.

Fig. Larva

The total larval period may vary from 11-29 days.

Pupa

The elongated comma shaped pupa is milky white in the beginning and turns brown. It is a nonfeeding stage lasting for about 6-10 days. The sexes are differentiated in this stage.



The total life cycle from egg to adult is reported to take about 20-36 days with average 26.75 days in *Ph. argentipes* in laboratory.

Taxonomic Considerations

There are 46 species of phlebotomine sandflies recorded in India which belong to two genera namely *Phlebotomus* and *Sergentomyia*. The genus *Phlebotomus* consists of eight subgenera representing 11 species.

Subgenus

Euphlebotomus

Phlebotomus

Paraphlebotomus

Synphlebotomus

Alderius

Larroussius

Anaphlebotomus

Idiophlebotomus

Ungrouped

Species

argentipes

papatasi & salehi

sergenti

eleanorae

longiductus

major major

colabaensis & stantoni

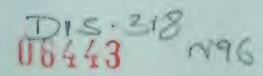
tubifer

newsteadi

(Often grouped with Euphlebotomus)



52



The genus Sergentomyia is represented by 6 subgenera and 35 species.

The identification of phlebotomines is essentially based on internal characters which are examined after processing the specimen, properly mounting on a slide and examination under microscope.

Processing & Mounting

Each field caught specimen of sandfly is to be kept in 5% KOH solution or soap solution for 24 hours. Alternatively, the specimen may be kept in 5% KOH and gently heated for about 4-5 minutes. This helps in removing the hairs, cuticular wax layer and viscera. The alkali/soap is then removed by thorough washing with ordinary water atleast thrice. The specimen is then dissected in mounting media, the head is oriented ventral side up to expose buccal cavity. The 8th abdominal pleura are stretched gently to expose spermethecae in females. In males, terminalia is properly oriented for examination.

Mounting Media

Hoyer's media is the most commonly used medium for sandfly mounting. It consists of following constituents:

Distilled water - 100 cc
Gum Arabic - 60 gm
Chloral hydrate - 400 gm
Glycerine - 20 cc

After mounting the specimen, it is left for 48-72 hours in an incubator/room temperature for making the cuticular structures clearer.

* Characters of Taxonomic Importance

Following characters are examined carefully for identification. It is desirable to follow a taxonomic key while attempting identification.

Head Capsule.

Head

- form of the head, relative length & width
- size and diameter of the eye and the distance between two eyes dorsally
- number and distribution of hair socket.

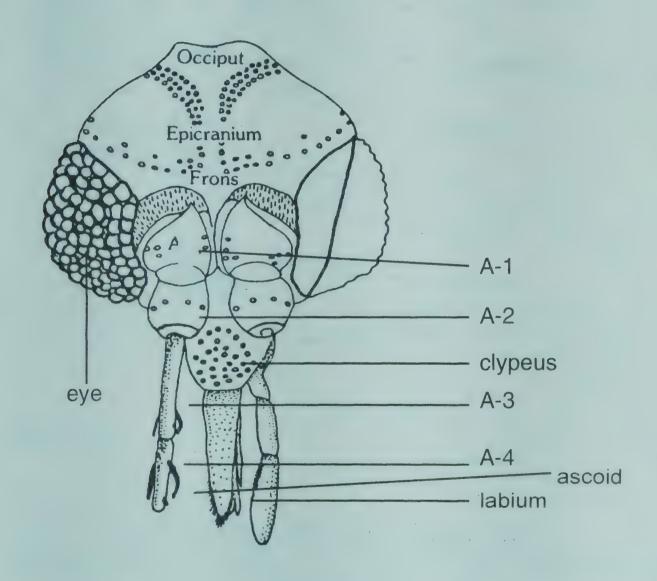


Fig. Head of sandfly (dorsal view)

^{*} Source: WHO SEA/VBC/35

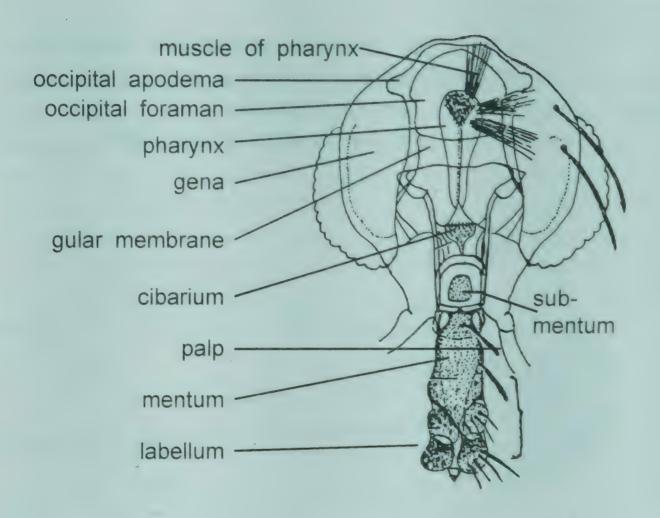
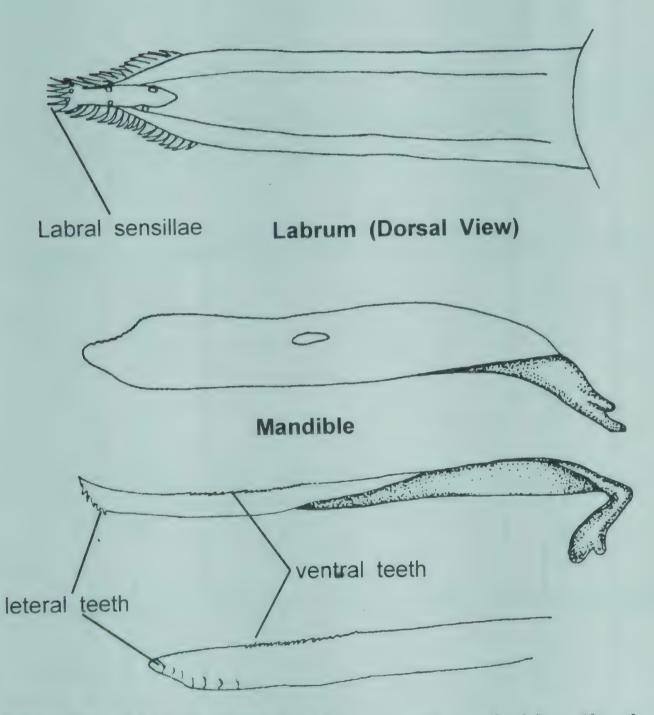


Fig. Head of sandfly (ventral view)

Mouth parts

- length of the labrum
- shape of the laberal tip
- shape of maxilla and number of various teeth
- shape of the mandible
- presence/absence of serrations on hypopharynx.



Maxillae (hook-tip above) Phlebotomus and ridge tip down Sergentomyia

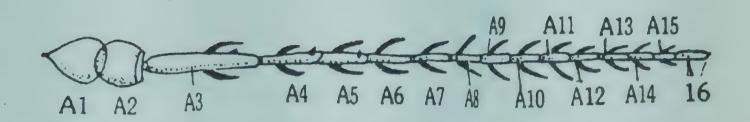


Fig. Hypopharynx

- length of the 3rd antennal segment (A3)
- relation A3/A4+5

Antenna

- relation A3/labrum (A3/L)
- antennal formula (number & position of ascoids)
- length of ascoid on A4 and relation 4/A4
- Papilla formula.



Antennal formula 2/3 - 1/6-15

Papilla formula 1/3-5



Antennal formula 1/3 - 15

Papilla formula 1/3-4

Fig. Antenna

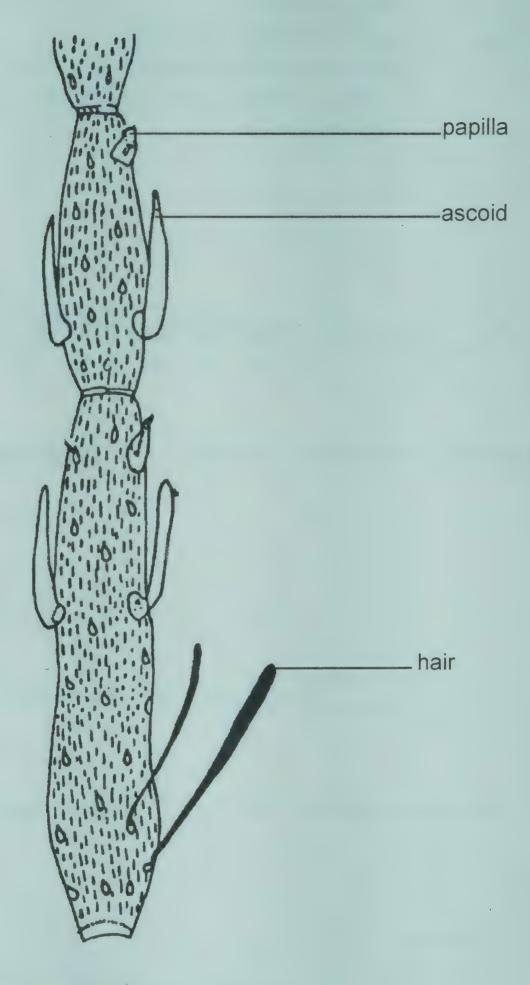


Fig. Antennal segments

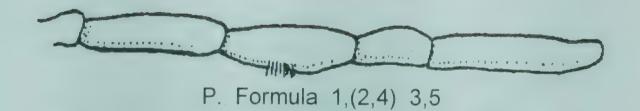
- Palpal formula
- relative length of palpal segments

Palpi

- palp length
- position of Newstead's scales.



P. Formula 1,5,4,3,2



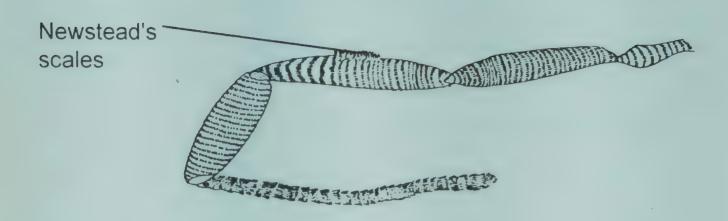


Fig. Palp common shape under the big magnification

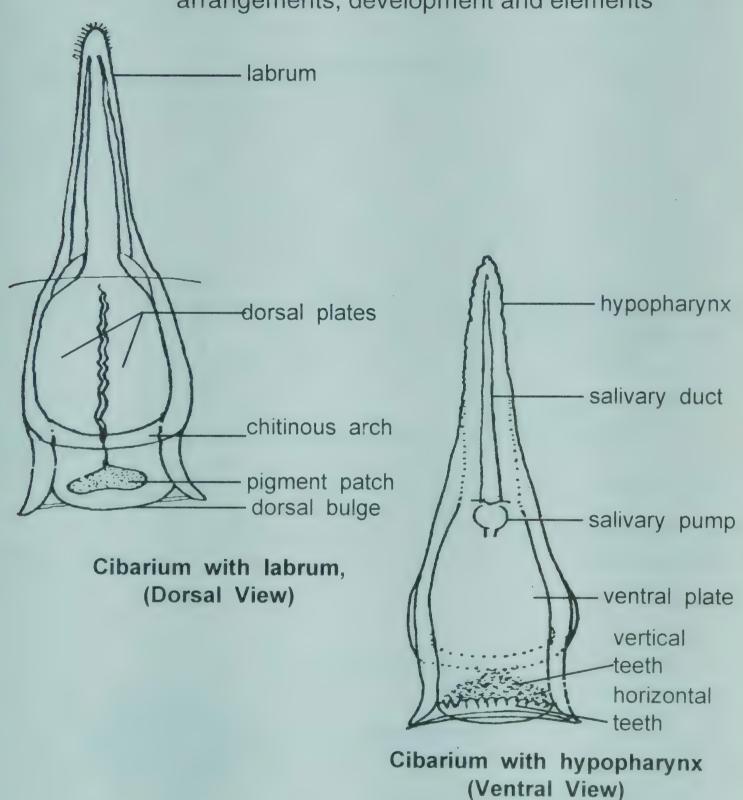


Fig. Newstead's scales or hairs

presence and number of horizontal and vertical teeth, position and arrangement

Cibarium

- presence, shape and colour of the pigment patch
- presence of the chitinous arch shape of the cibarium & ventral plate
- presence and number of dorsal bulges; arrangements, development and elements



Ciborium

Pharynx

- shape of the pharynx, length & width
- Pharyngeal armature.

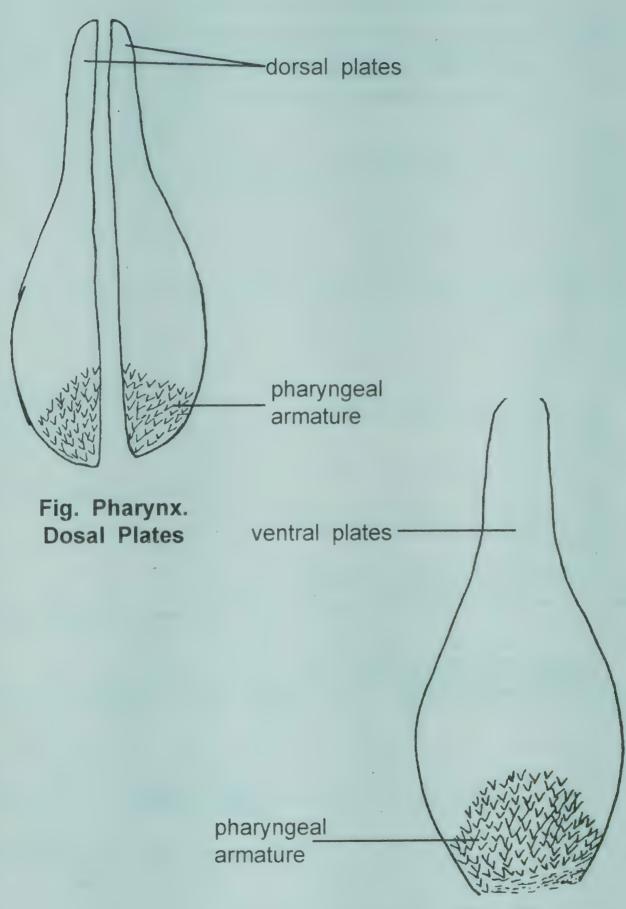


Fig. Pharynx. Ventral Plates

Thorax

- length of mesonotum
- pigmentation of different sclerite
- pleural hairs.

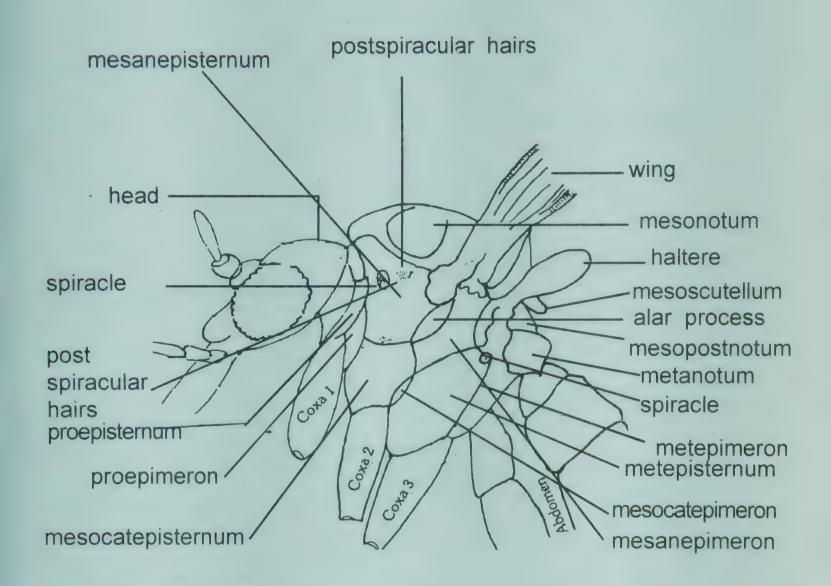


Fig. Thorax

Legs

- length of hind leg and relation with wing length
- length of the femur, tibia and each tarsal segment of hind leg and relative relation in length of each
- short spines on femora.

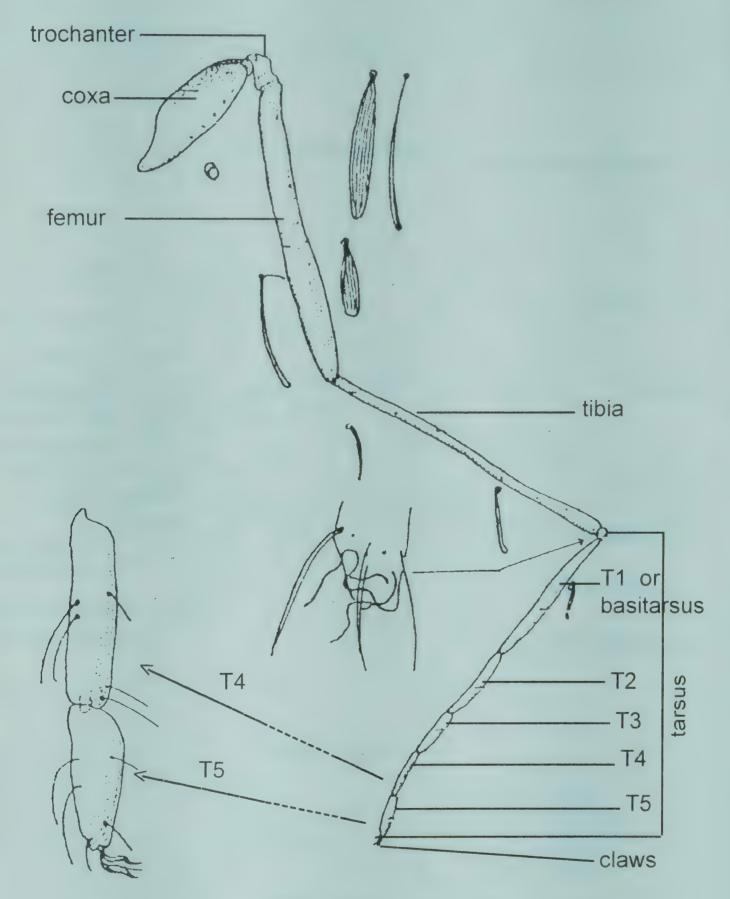


Fig. Leg

Wings

- length and width of the wings and their relation
- length of R2(α), R2 + 3 (β) and their relation
- relation R2/R2+3(γ/β) or wing index
- length R2+3+4 (γ) and R1 (δ)
- distance between R2+3+4 and M1+2 (π)

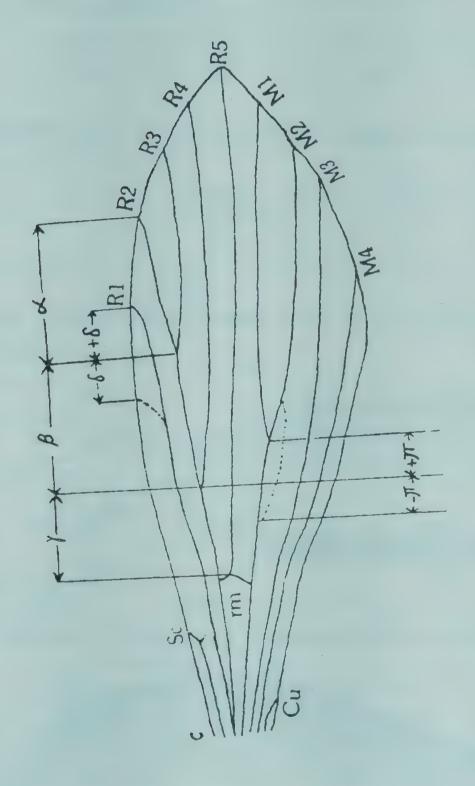


Fig. Wings

Abdomen

- presence or absence of the erect or recumbent hairs on tergites 2-6
- pigmentation of tergites.

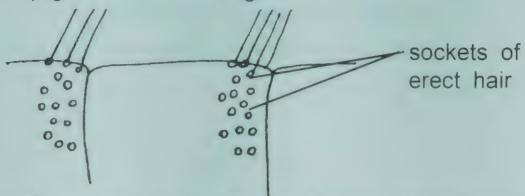


Fig. Abdominal tergites (1-4) with erect hair (Phlebotomus)

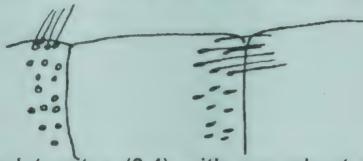


Fig. Abdominal tergites (2-4) with recumbent hairs (Sergentomyia)

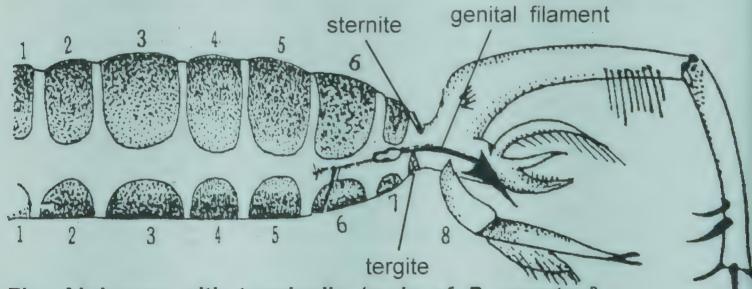


Fig. Abdomen with terminalia (male of P. papatasi)

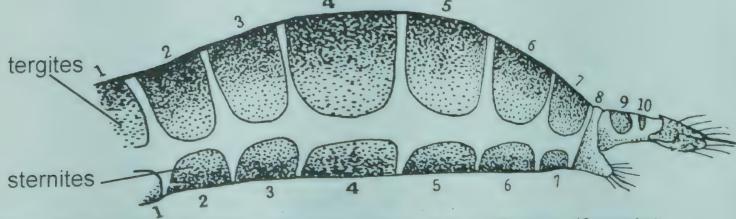


Fig. Abdominal tergits sternites and appendages (female)

Male terminalia

- length of each appendage and relative lengths
- presence, size and shape of the basal process
- Number and position of hairs on coxite
- Number, length and position of the spines on style and process on parametre
- shape of the aedeagus
- presence of spines at the end of surstyle
- shape of the genital filaments and genital pump.

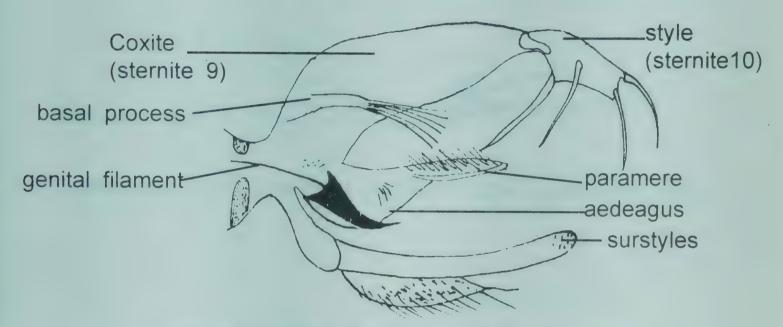


Fig. Terminalia of male (lateral view)

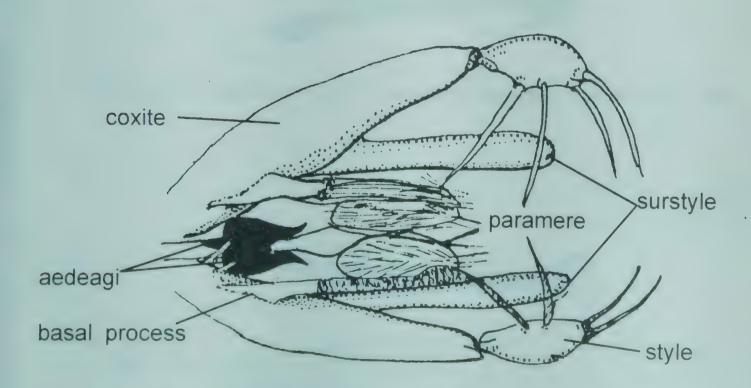


Fig. Terminalia of male (dorsal view) (P. sergenti)

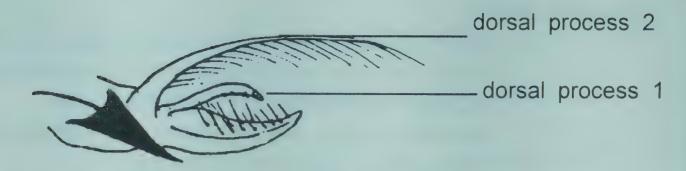


Fig. Paramere with 2 dorsal processes (P. papatasi)



Fig. Paramere with 2 ventral processes (P. argentipes)

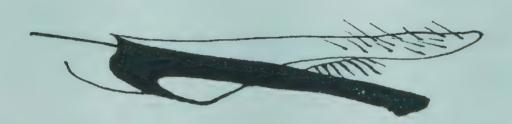


Fig. Paramere without process long aedeagus (Adlerius)



Fig. Genital pump

Spermethecae (Female)

- shape
- length of the postgenital plate
- shape of the furca.

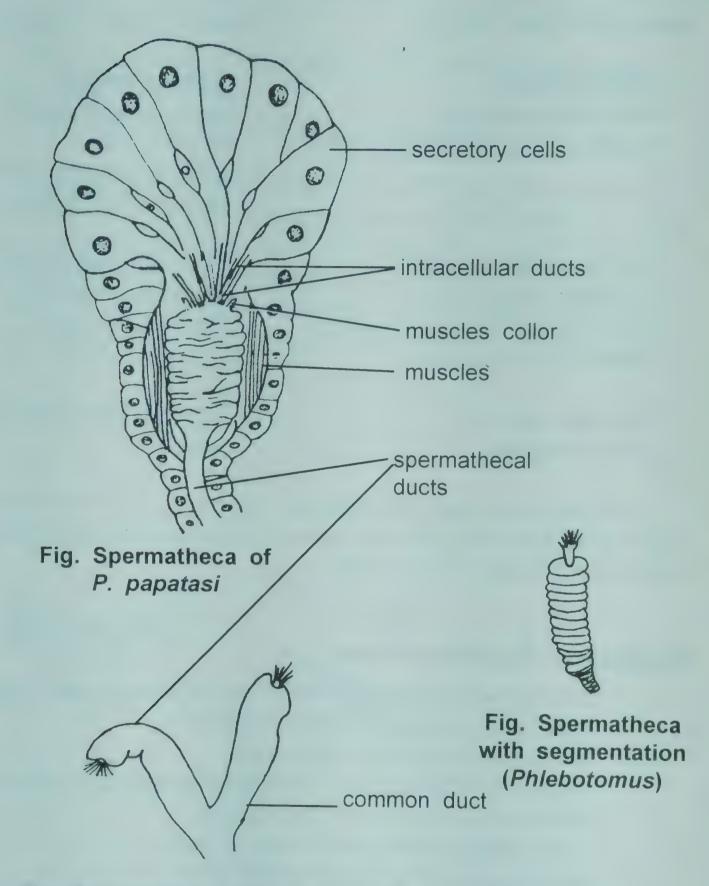


Fig. Spermatheca without capsule (Sergentomyia)

It is advisable to follow a taxonomic key by paying attention to above mentioned characters of taxonomic importance for proper species identification, which is given subsequently. For convenience specific character combinations for broad classification of two genera are given below:

Phlebotomus

- Hairs on abdominal tergites 2-6 uniformmally errect
- Cibarium without teeth,
 if present, only in the
 form of minute spicules and
 never in a definite row.
- Pigment patch is always present

Sergentomyia

- Hairs on abdominal tergites 2-6 with recumbent or with very few errect hairs.
- Cibarium teeth and denticles usually in transverse rows
- Pigment patch always absent.

To facilitate quick identification of VL vector *Phlebotomus* argentipes, the characters of subgenus *Euphlebotomus* and species argentipes are also given below:

Subgenus Euphlebotomus

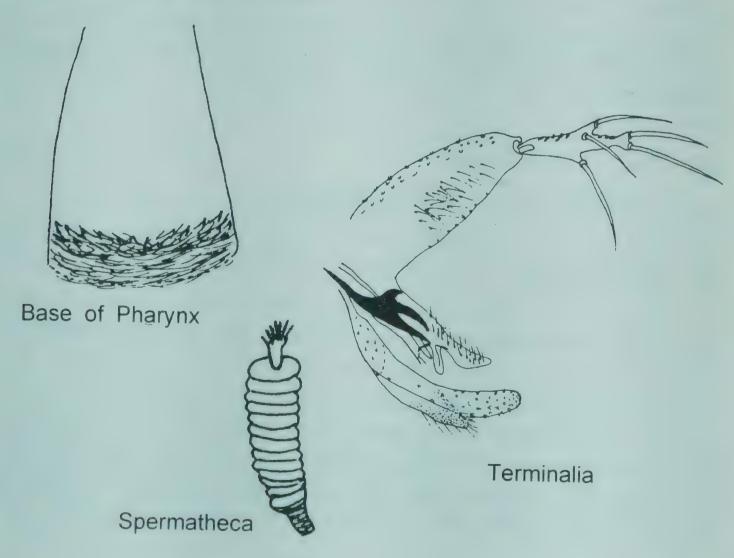
- Cibarium with spicules or unarmed, Pharynx usually armed. Palps extending further than antenna 3.
- Terminalia shorter or middle size.
- Parametre with short 1-2 ventral processes or a tubercle.
- Style with 5 and rarely 6 long spines.
- Coxite without setae and basal process.
- Aedaegus short with long lateral spine.
- Spermetheca with regular segmentation and head of spermetheca with a distinct neck.

- Apical segment of spermetheca enlarged or separated from others by more deep furrows.
 - Spermetheca with more than 15 segments.

Species - argentipes

- Paramere with 2 long ventral processes and long distal parts. Coxite wide.
- Spermetheca short with 15-17 segments.

Though specific identification of sandflies has to be done under microscope, *P. argentipes* can be screened alive by looking for their silvery white legs, particularly while carrying out susceptibility tests. Presence of brownish / dark brown hair on thorax also helps in broad screening of *P. argentipes*. However, after the test is over, all the exposed sandflies must be processed, mounted and examined for confirmatory identification.



ENTOMOLOGICAL TECHNIQUES

1. Adult sampling techniques

Of the various methods available for adult sampling, the selection depends on the objective of the sampling, biotope selected for sampling and the limitations of a particular technique. Most commonly used techniques are summarized below:

a) Hand Collection

This is the most common method wherein sandflies sitting on a surface are caught with the help of an aspirator or test tube and a torch light. This method is particularly useful for longitudinal monitoring of manhour densities. However, in sandfly collection the ordinary mosquito-barrier netting between glass tube and rubber tubing of the aspirator must be replaced by a muslin cloth as the smaller size of sandflies enable them to escape through ordinary mosquito net.

b) Trap Collections

Usually 4 types of traps are used:

i) Sticky trap

This is the most extensively used trapping devise wherein sandflies are trapped in a layer of castor oil. Suspended arched sticky papers/foils of standard size (20 x 30 cms) are placed at a height of about 4-5 cms from ground with convex sticky side towards ground. Traps are usually laid in the evening and collected on following morning. Sandfly density per trap is calculated for comparisons. Sticky traps are particularly useful in collecting sandflies from hidden shelters like burrows, cracks, tree holes, etc.

For some species showing repellency to castor oil, other vegetable oils are required to be used. However, in India these can be safely used against *Ph. argentipes*.

ii) Illuminated Sticky traps

Box shaped batteries are hung on the walls facing sticky traps to make them illuminated. In some studies, these traps have provided higher catch as compared to ordinary traps.

iii) Light traps

CDC miniature light traps are often used for sandfly collections. However, nylon mesh cage suspended in a rigid frame are better than the collapsible cages provided with the traps. Further, for sandflies they are modified to give UV light or white light.

iv) Funnel traps

These are particularly useful in collecting flies from rodent burrows. Traps are placed just at the mouth of the burrow to catch the flies emerging out of burrows. The inner side is provided with sticky paper or foil.

Other traps used in mosquito collections like double bednet, stable net, malaise trap, magoon trap, etc. can also be used but the effectiveness is not yet well demonstrated.

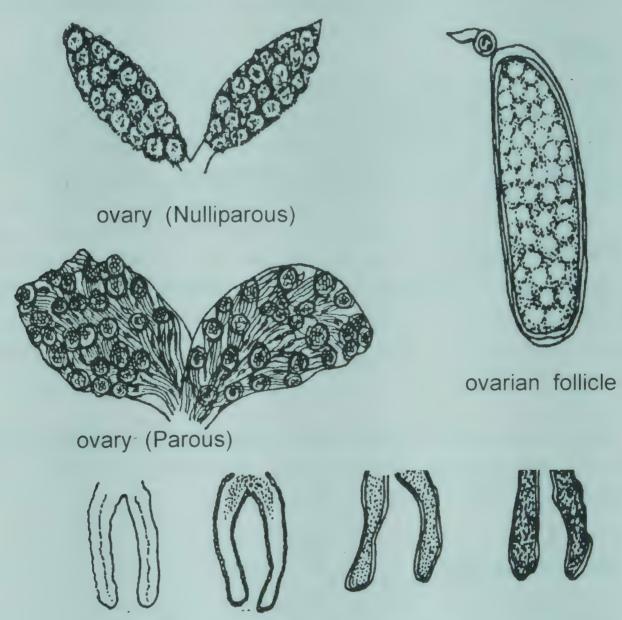
c) Bait collections

Both human and animal baits can be used. However, the fact that sandflies are well known for their patchy distribution must be kept in mind while designing bait sampling. Due to clustering habit of sandflies, bait sampling must be extended to cover all parts of a village.

Age Determination

Usual method of age determination of sandflies is the examination of ovariole relics. The ovaries are dissected in sterile saline and the ovarian follicles are examined for dilitations. Each relic represents one genotropic cycle.

The examination of accessory glands for secretary grannules also provides criteria for determination of age (parity).



accessory glands with graunles at various stages of ovariole development

Host Preference

The blood meal of a freshly fed sandfly is sampled on a filter paper which may be subjected to precipitin test, Gel-diffusion technique or ELISA to determine the source of blood meal.

Vector incrimination

After dissecting a sandfly in sterile saline, midgut is examined for presence of flagellates. If found positive, head should also be dissected for examination of cibarium, pharynx and proboscis. The promastigotes must be spread on a slide, fixed with methanol and stained with Giemsa or Leishman stain. The presence of promastigote, however, does not confirm the species of the parasite as all promastigotes are morphologically indistinguishable. For confirmation, samples should either be subjected to xenodiagnosis or to biochemical characterization of parasite.

For incrimination of vector, Barnett, Killick - Kendrik and Ward have suggested following criteria:

Criterion 1: Feeding habits

A vector must be anthropophilic and also feed on the reservoir host(s) [In case of VL in India - Man]. Baited trap collections followed by blood meal analysis provide information about anthropophilly.

Criterion 2: Carrier of the parasite

Seeing a flagellate in a wild caught sandfly is not an adequate proof for incrimination and repeated occurrence of parasite infection needs to be demonstrated. For such an attempt, older populations of sandflies that are definitely available towards the end of the season, must be sampled and dissected / processed for detecting presence of parasites

Criterion 3: Distribution

A vector has a distribution which agrees with disease distribution but certainly is wider than the disease itself.

Criterion 4:- Vector competence

A vector readily supports the development of the parasite and efficiently transmits the parasite by its bites. Thus, for incrimination studies on taxonomic relation of VL vectors, epidemiological evidence, natural infection rates of sandflies and identification of promastigotes from sandflies are required.

Determination of susceptibility to insecticide

The conventional WHO susceptibility test kit must be used. Freshly fed *Ph. argentipes* can be subjected to preliminary screening on the basis of silvery white legs. However, after recording the data, all sandflies, subjected to test must be examined under microscope after mounting and due corrections be made in the observations before interpreting the results.

Sampling of immature stages

Sandflies breed in cracks, crevices and other places with soils rich in organic contents. The resemblance in soil and larval coloration makes it difficult to detect larvae visually in their habitat. The soil is collected, kept in a petri dish and then examined under microscope (40 x magnification). To facilitate screening of larger soil samples, a floatation technique is often practiced. The soil samples are immersed in a saturated sugar solution i.e.3 parts sugar + 5 parts water. Larvae and pupae float in this solution. These are then passed through a series of sieves and finally the residues are examined under the microscope.

VISCERAL LEISHMANIASIS CONTROL IN INDIA

Visceral leishmaniasis, once highly endemic in several parts of the country with epidemic periodicity, declined to the levels of virtual eradication in sixties as a collateral benefit of mass DDT spraying undertaken since 1953 under NMCP and since 1958 under NMEP coupled with effective treatment through kala-azar clinics. Though there was virtual disappearance of fresh Kala-azar cases in late sixties, parasitic reservoir continued in the community in the form of Post Kala-azar Dermal Leishmaniasis.

With the entry of earstwhile attack phase areas under NMEP into maintenance phase since 1961 onwards with consequent withdrawl of DDT spraying, sandflies began to build their densities slowly in the absence of any insecticidal pressure. This increase in vector populations coupled with persistent parasitic reservoir in the community (PKDL) led to establishment of indigenous transmission. By 1974, 4 districts of Bihar namely Vaishali, Muzaffarpur, Samastipur and Sitamarhi became endemic for kalaazar which spread to adjoining areas.

Though a Control Programme with UNDP assistance was launched in 4 districts by NICD in 1977, it was withdrawn in 1979 after only a marginal reduction in morbidity and mortality due to kalaazar.

Until 1989-90, kala-azar control activities were being undertaken by the States on focal basis by diverting NMEP resources. However, during 1990-91, Planning Commission approved a centrally sponsored kala-azar control scheme which is in operation in both the endemic states namely Bihar & West Bengal.

Strategy

A three pronged strategy is being implemented for control of visceral leishmaniasis in India.

- i) interruption of transmission through vector control,
- ii) early diagnosis and complete treatment,
- iii) Information Education Communication.

To implement this strategy a detailed district action plan has been worked out indicating various activities, levels of implementation, implementing agencies, responsibility and accountability. A model district action plan is given in Annexure.

i) interruption of transmission through vector control

Two rounds of DDT indoor residual spraying is being undertaken in kala-azar endemic areas at the dosage of 1 gm per sq. metre on all indoor surfaces upto 6 ft. from ground level. Spraying upto 6 ft. is a technically cost effective measure in view of hopping nature of the sandflies. The spray seasons are:

	First round	Second round
Bihar	Feb-March	May-June
W.Bengal	May-July	August-Oct.

Spray operation conducted during Feb-March and May-June are operationally feasible and technically effective in view of two peaks of *P.argentipes* densities in Bihar i.e. March / April and August/September and financial year ending. Further spraying during May-June ensures completion of spraying before monsoons which sets in July and the majority of north Bihar areas being flood prone, restricts the accessibility. Change in spray season in West Bengal to synchronize with Bihar has also been proposed. Modalities of spray operations are discussed subsequently.

ii) Early diagnosis and complete treatment

For early diagnosis both active and passive surveillance have been envisaged. For active surveillance, periodic case searches are to be organised.

For passive surveillance PHC serves as the basic unit in the rural areas and hospitals as the II level units followed by Medical College hospitals.

For ensuring complete treatment, a treatment card system has been introduced. The treatment cards have been printed in 3 colours.

Green - to be issued for I course of SSG by the treating physician after diagnosis.

Red - to be issued for II course SSG treatment by the Medical officer i/c of PHC to the cases unresponsive/partially responsive to I course of SSG.

White - to be retained as record copy of the treatment centre to facilitate monitoring and supervision.

The treatment for SSG unresponsive cases has to be available at the district hospital. On production of treatment cards indicating details of SSG treatment, the hospital physician may admit the patient for diagnosis and treatment with alternative drugs

iii) Information Education Communication

I.E.C. is the most crucial component of the strategy and success of any programme largely depends on effectiveness of IEC campaign. In kala-azar control, due priority has been assigned to this component. All probable approaches must be exploited e.g.

- inter- personal communication through primary health care infrastructure.
- Printed publicity like pamphlets, advertisements, folders, booklets, etc.
 - Audio visual communication channels
 - Orientation of opinion leaders, etc.

Further a "Kala-azar Fortnight" may also be observed with effective IEC inputs and simultaneous detection and treatment campaign. Tentative work plan could be:

- Microplanning for door to door search, treatment camps etc. as envisaged in model district action plans.
- Proper Logistics input like supply and stocking of survey forms, reports/returns, diagnostic facilities, drugs and treatment cards at sub-centres etc.
- Systematic IEC campaign involving appeals from well known social, political, administrative and professional personalities, publicity regarding calendar of activities, location, dates and timings of diagnosis and treatment camps, importance of such campaign, knowledge about signs, symptoms, diagnosis and treatment, advance intimation seeking community co-operation etc.
 - Meticulous conduct of activities as per micro plan.

A systematic approach with constant persuation and continued efforts helps in enlisting community support both in terms of acceptability of Governmental efforts as well as involvement of community in kala-azar prevention and control activities. It is also important to remember that IEC can not be successful as one time activity. Involvement of Community and continuity of efforts is important in making it effective and successful.

Vector Control Operations for Kala-azar Control in India

1. Insecticide used DDT 2. Dosage 1gm/sq.metre Formulation for spray 3. 5% suspension Preparation of formulation 4. measure 1.5 kg (3 lbs) DDT 50% wettable powder and 15 litres (3 gallon) water. Mix DDT thoroughly with small quantity of water to make a homogenous paste. Mix all the water to make 15 litres suspension. Stir well and filter. 5. Spray pump Stirrup pump Discharge rate of nozzle 650-750 ml/minute. Squad composition 6. Superior Field Workers - 1, Field Worker - 5 Equipments per squad Stirrup pump Two Bucket 15 litres - Four Bucket 5/10 litres - One Spare nozzle One Asbestos thread -3mt. Pump washers - Two Measuring mug - One Strainer cloth - 1 mtr Plastic sheet $(3 \times 3 \, m)$ - One Soap - One **DDT** requirement 7. 75 MT 50% wdp per million population for 2 rounds spray.

8. Output per squad

- Over 80 houses per day
- 9. Advance spray programme
- As per district action plan
- 10. Advance intimation card
- As per district action plan

For kala-azar control spray operations, a squad is supposed to get signatures of village mukhia on his daily diary as a mark of completion of his work in a particular village.

Planning of Spray Programme

The spray programme planning must be undertaken in such a way that the programme as per the format is finalised at atleast one month before the scheduled date for commencement of spray. During this month the programme must be circulated to all concerned both upwards i.e. State level officers, Zonal Officers, District Magistrate, etc. and down wards i.e. Medical Officer PHC, Block Development Officer Village Mukhia, etc. The DDT must be stocked at identified places and recruitment of seasonal staff to be completed. It is often seen that the training aspect of seasonal staff and peripheral supervisory staff is not accorded with due priority. This is a crucial activity and for kala-azar control, ample emphasis has been laid on orientation training. Even if a person is repeatedly being recruited for spray operations every year, it is in the larger interest of the programme to provide him an orientation training before sending him in field. The video cassette made for this purpose must be used. The same is true for other peripheral supervisory staff i.e. MPHW, BHW, BHI, MI, Health Supervisor, etc. For improvement of supervisory skills of PHC and district level officers, Dte. NMEP initiated a series of orientation training courses in methods of supervision and evaluation of kala-azar control activities. Officers up to PHC level trained in these courses are required to train staff working with them.

SUPERVISION

Supervision is a critical activity that decides the level of effective implementation of any strategy. Further, supervision should not be considered as an exercise of finding the fault in implementation, rather it is an exercise to strengthen programme implementation.

Supervision in true sense must be taken up as a responsibility for identifying the lacunae and rectifying them for improvement in programme performance. Therefore, it should be taken up as a supportive activity.

For effective implementation of a strategy, all strategy components and associated activities right from planning stage need to be supervised. Further, the concept of supervision and methodology as well as skills must be developed in all supervisors. Needless to mention that supervision does not restrict to national, state, zonal or district levels only, it has to percolate right upto the periphery through various levels of programme implementation.

Types of Supervision

The supervision may be done by adopting either of the following ways in isolation or combination:

a) Indirect Supervision

Supervision through examining reports, returns and collecting informations through various channels but not visiting the work area could effectively be used to draw inferences about status implementation of an activity and areas for strengthening.

b) Direct Supervision

Supervision done by visiting the work area and witnessing the performance could be labelled as Direct Supervision. Further, both

indirect and direct supervision can be undertaken "Concurrently" during the activity or "Consecutively" after completion of the activity.

Major areas for supervision in kala-azar control include -

i) Supervision of planning

Planning in kala-azar control operations in India is a decentralized planning that initiates from periphery. PHC wise plans are to be prepared in the format given in model district action plan. These are to be compiled and consolidated at district level. Timely development of such plans can be ensured through effective supervision.

ii) Logistics

Supervision of logistics is of utmost importance. Any lapse may lead to failure of entire operation. Each and every component, no matter how minor it appears to be, needs to be supervised closely.

iii) Surveillance

Regular periodic supervisory visits help in improved surveillance both passive and periodic search and also helps in determining the effectiveness of reporting system.

iv) Diagnostics and Treatment

Major areas that require attention while supervising diagnostics and treatment services include availability and quality of diagnostics facilities, competence of technical staff, awareness of medical officer regarding diagnostic criteria, treatment schedule, criteria for cure etc., availability of drugs, maintenance of records, treatment cards etc. One of the crucial factors requiring special attention of supervisor is the patient reporting, compliance. and treatment failure rates.

v) Vector control

Supervision of spray operation must be of concurrent and consective nature through all echelons in the organisation. All the reports and returns on spray operation are of no value unless these are substantiated by field visits. It is true that the supervisory staff cannot possibility visit every room in every house in every village. But they should be able to take a stratified sample. Normally the sample is highest in respect of the peripheral supervisors and become inversely proportional to the upward ranking in the hierarchy. Officers working at every level of programme implementation must be involved in supervision with a given target and every officer at one step above in the heirarchy should invariably supervise the operations including the discharge of supervisory responsibilities by his subordinates.

(1) Concurrent supervision and observation to be made

Assessment of work at the time of actual spray operation is of utmost importance However, it is well known that during inspection every worker tries to put up his best show and thus supervision is effective when it is undertaken as a surprise visit. It provides information about skills and sincerity of spray workers as well as qualitative and quantitative performance of spray personnel. At the same time, this is necessary as it will be important to know if the spray squad are conversant with their job and are capable of ensuring thorough coverage, have been trained properly on various aspects of the operation like preparation of insecticide suspension, supervision, determination of discharge rate, speed of spraying etc.

The following should be checked during such supervision:

- Date of advance notification and the correct maintenance of time table for spray operation.
- Turn out of spray squads.

- Condition of the spray pumps and nozzle tip discharge rate.
- Preparation of suspention.
- Actual spraying operation including the technique, speed and coverage, etc.
- Extent of actual refusal to accept spray and locked houses.
- Maintenance of records by the S.F.W.
- Consumption of insecticide as determined by the quantity issued and stock in hand vis-a-vis coverage
- Frequency of checking of the squad by inspectors and other supervisory personnel and remarks, if any.
- Arrangement for "mopping" up.

(2) Consecutive supervision

Supervision after completion of spray either on the same day, previous day of week or earlier is consecutive supervision.

The following is required to be checked:

- Evidence of insecticide deposit in every sprayable surface.
- Dispersal of the deposit.
- Evidence of recent spray.
- Number of room in each house (taken as sample), sprayed satisfactorily, partially and unsprayed.
- Percentage of refusal and locked houses.
- Factors responsible for high refusal rate, if any, and action taken.
- Attempts made for "mopping up" operation when refusal rate is high.
- Extent of mud plasteing on the walls, if any, and other relevant matters.
- Correlation between actual coverage and data presented.

At the time of conducting the supervision, observations must be recorded in detail so that quantitative information becomes available on all aspects of spray operations including quality, extent of coverage, insecticide handling, storage, consumption, community response etc.

(3) "Mopping up" Operation

The expression is used for spray squads following the main team in order to ensure that the houses left unsprayed by the previous squad due to one reason or other, are attended to. This is one of the most vital parts of the campaign when the refusal rate is high or when at times, large number of houses are found to be locked.

vi) IEC and training

IEC is an important area that requires close supervision. Every step involved in development of IEC prototypes/modules campaign planning etc. need to be supervised for ensuring communication of the correct and target oriented information. Further, dissemination of information has to be ensured. All IEC activities must be evaluated particularly in terms of their effectiveness in enlisting support of the target group.

Training is another critical activity that must be supervised very closely at every step as any mistake in developing target oriented module, selection of trainers with skills of communication and the quality of training material is bound to affect adversely the effectiveness of such an activity and shall have serious consequences.

For effective supervision, guidance and monitoring of kalaazar control activities, block level, district level and state level committees have been constituted in Bihar.

Responsibility and Accountability

In Bihar, an exclusive infrastructure within the primary health care system has been identified and made responsible and accountable at various levels of programme implementation.

- 1. National level
- Dte. of NMEP for planning, monitoring, procurement and supply of drugs and insecticides, supervision, training, evaluation, technical guidance and policy formulation, etc. Regional Office for Health & FW also assist in supervision and liaisoning with States.

- 2. State level
- Chief malaria officer/State Programme Officer for planning, implementation, monitoring, logistics, supervision, training and evaluation. Civil Surgeon Diagnosis & treatment.
- 3. District level
- Civil Surgeon is overall responsible and accountable for all Kala-azar control activities in the district. District Malaria Officer (with the assistance of Assistant Malaria Officer) for district plan, spray programme logistics, spray operations, supervision, evaluation, IEC, etc.

- 4. PHC level
- Medical Officer i/c overall responsibility.

 I Medical Officer (designated as Kalaazar Medical Officer in Bihar) over all responsible and accountable for all kala- azar activities in PHC through Inspectors/B.E.E., etc.

- 5. Sub-Centre Health worker/ANM responsible and accountable for Kala-azar activities.
- 6. Hospital level Medical Suptd. responsible and accountable for diagnostic and treatment services.

For over all supervision, guidance and monitoring, district and block level committees are also functioning in Bihar. Further, to involve sense of responsibility and accountability for improving reporting of cases and implementation of control activities, Government of Bihar declared kala-azar as a "Notifiable Disease" through a special Gazette notification issued in November, 1991.

FOR CONTROL OF KALA - AZAR

MAJOR ACTIVITIES

- 1. CASE DETECTION AND TREATMENT.
- 2. VECTOR CONTROL: INSECTICIDAL SPRAYING
- 3. INFORMATION EDUCATION COMMUNICATION

IMPLEMENTATION PLAN:

ACTIVITY	LEVEL	TASK	MODALITY
1	2	3	4
1. Case dection			
Passive case detection	PHC/Addl.PHC Referal Hospital sub Divisional Sadar Hospital District Hospital	Diagnosis Clinical & Lab confirmtion	The Health workers working in the field to guide suspected cases to the centre for examination by the Medical officer who is to diagnose & initiate the treatment.
Active case detection (Search & survey)	Village/ sub-centre	Identification of cases, Diagnosis & Initiation of treatment.	Team of Medical Offficer / Lab Personnel to visit camp for diagnosis & initiation of 1st line treatment

TIME FRAME	RESPONSIBLE AGENCIES	MONITORING AGENCIES	REMARKS
5	6	7	8
Through out the year.	Peripheral worker Technician Medical Officer	MO i/c PHC / Civil Surgeon	
Nov. to Jan.	Medical Officer Survey Team / Medical officer i/c PHC	M.O.i /c PHC Civil Surgeon	

ACTIVITY	LEVEL	TASK	MODALITY
1	2	3	4
Mopping up 2. Treatment	Village / sub centre	Follow up of cases detected during survey as well as fresh case, if any	The Doctor with the help of sub centre i/c to organise follow up activities for the task indicated in the previous column.
I Line Treatment SSG I Course	Village/ Sub centre (active) PHC (Passive)	Administration of SSG daily for 20 - 30 days as per prescription based on drug policy.	The treatment card to be issued by M.O. on confirmation of cases along with administration of first dose, subsequent dose to be administered by the sub-centre staff with due entries in treatment card. The M.O PHC to administer the dose on particular week day of his visit to the sub centre along with examination of the patient.

RESPONSIBLE AGENCIES	MONITORING AGENCIES	REMARKS	
6	7	8	
Medical officer PHC Leave re- serve etc (Survey team)	M:O.i/c PHC Civil Surgeon		
Subcentre staff M.O. PHC	Medical Officer PHC concerned with subcentre and M.O. i.e Medical Officer PHC / Civil Surgeon.		
	AGENCIES 6 Medical officer PHC Leave reserve etc (Survey team) Subcentre staff M.O. PHC	AGENCIES 6 7 Medical officer PHC Leave reserve etc (Survey team) Subcentre staff M.O. PHC Medical Officer PHC concerned with subcentre and M.O. i.e Medical Officer PHC / Civil Surgeon.	

ACTIVITY	LEVEL	TASK	MODALITY	
1	2	3	4	
SSG IInd Course	P.H.C./Addl. PHC	Confirmation of completion of first course, requirement of repeat course, administration of repeat course SSG	The Patient not responding to the first course to be re examined by the MO for necessity of second course on production of card and to be issued with different colour treatment Card specifically meant for second course and the course to be given by the PHC / AddI PHC	
Il Line Treatment (Pentamidine Isethionate)	Sadar Hospital / District Hospital	Identification of cases unresponsive to given two courses of SSG and treatment with pentamidine follow up.	The Patient not responding to even 2 courses of SSG to be referred to Sadar Hospital by the M.O. i/c with due certification. The patient to produce both the cards indicating administration of 2 courses of SSG. After examination, if pentamidine considered necessary, indoor treatment to be given. Both the cards of SSG treatment to be kept as record. Feed back of treatment of patient to respective PHC & sub centre to be given by the Hospital for future reference and follow up.	

TIME FRAME	RESPONSIBLE AGENCIES	MONITORING AGENCIES	REMARKS	
5	6	7	8	
Round the year.	Medical Officer/ PHC	Civil surgeon.		
Round the year.	Civil Surgeon Sadar Hospital	Distt. Magistrate / C.M.O. / State Level Officer		

ACTIVITY	LEVEL	TASK	MODALITY
1	2	. 3	4
Illrd Line Treatment Amphotericin B	Med. College Hospital / Institute	Identification of cases unresponsive to both I & II line drugs.	Treatment as per drug policy to be administered under strict medical supervision
3 - Vector control	Village / Panchayat	Indoor DDT spraying in all cattle & human dwelling up to 6ft height from ground at the rate of 1 gram per square metre.	Spraying to be undertaken by seasonal spray workers. DDT to be stored at Panchayat / Subcentre / appropriate storage place, latest by 31 Jan. Panchayat Mukhiya & Sarpanch to be involved in spray activities and for ensuring 100 per cent coverage. squad to obtain signatures of village Mukhia etc. as a mark of completion of spray work in the village

TIME FRAME AGENCIES	RESPONSIBLE AGENCIES	MONITORING	REMARKS
5	6	7	8
Round the year	Civil surgen / Sadar Hospital / Institute	Distt. Magis- trate CMO/ State level Officer	
Feb. March May - June (as per spray schedule)	Malaria Inspector Basic Health Worker MO PHC concerned AMO / D.M.O.	C.S. / Distt. Magistreatse state level officer	

ACTIVITY	LEVEL	TASK	MODALITY
1	2	3	4
4- Health Education	Village / Home.	Advance intimation about search and spray programme; information regarding sign symptoms treatment facilities, importance of complete treatment. Do's and Dont's	Mass media, Panchayat notification, inter personal communication through peripheral workers; Adult education centres; B.D.O./ Gram Sevaks' Circular to school, use of Folders, Pamphlets. Advance intimation
5. Training Peripheral workers.	PHC	of spraying etc. Orientation regarding disease presentation guidance to the patient & follow up. spray operation etc	Cards etc. On the monthly meeting day Orientation to be arranged by MO i/c PHC.
Survey Team	Dist.	To orient for active case detection.	2 days training including field visit to LRP MOs/ MO PHC, Tech. etc
Medical Officers.	Dist.	5 days intinal, refresher one day, on diagnosis treatment & control.	Training on all aspects of kala-azar

TIME FRAME AGENCIES	RESPONSIBLE AGENCIES	MONITORING	REMARKS
5	6	7	8
October for advance information for KA Fortnight / active search and year round activities; for advance intimation prior to spray as per schedule, education. Throughout the year	D.E.E. Health Inspector B.D.O. Gram	D.M.O. /C.S. / D.M.	
1st week of Oct. and before spray represper periodecally	M.O.i/c PHC	D.M.O. / C.S.	
October	CS/DMO	CS/DMO D.M. D.M.O. /CS	
Throughout year	CS/DMO	D.M./CS	

ACTIVITY	LEVEL	TASK	MODALITY
1	2	3	4
Spray super visors & opinion Leaders	PHC/Panchayat	Qualitive & Quantitative spraying Technique, demonstration.	Demonstration training. on effective & proper spraying
Media Personnel / Opinion leaders.	Dist.	Orientation toward problem & communication priorities.	Briefing one day.

Supervision

Orientation training in methods of supervision covering all aspects to be organized for at least 5 days for PHC, District, Zone & State level officers.

In addition to personnel individually identified at various levels of implementation, block level, subdivisional and district level kala - azar control implementation committees to supervise and undertake monitoring and advisory work through out the operation. Further, surprise checks and independent evaluation to be done by district and state Level officers as well as national nodal organization.

Flow of Information

A regular weekly information monitoring to be done at the PHC level, District level and State Level for all the activities scheduled for specific time and period with consquent feed back to the centre. The channel for flow of information should be as follows:

TIME FRAME AGENCIES	RESPONSIBLE AGENCIES	MONITORING	REMARKS
5	6	7	8
Monthly meeting day round the year	DMO/CS	D.M.	
October / Jan	DMO/CS	DMO /CS	

Informations regarding treatment and search at the PHC level by the Medical Officer incharge PHC to be sent to Civil Surgeon / DMO. The C.S. / DMO will compile the information gathered from all the PHCs and send weekly report.

Information regarding DDT spray will be compiled at the PHC level by the Malaria Inspector and will be counter signed by the M.O. i/c PHC and sent to the district Malaria Officer every week. The District Malaria Officer in turn will compile the reports of all PHC together and send it to the Chief. Malaria Officer / Programme officer.

Any failure in regular feed back should be the personal accountability of officials at different levels.



KALA-AZAR CONTROL ACTION PLAN MODEL CALENDAR OF ACTIVITY FOR A PHC

Remark 14 Control 13. Vector 12. rame Mopping survey dn YEAR: Search I.E.C. Active 10 0 Name of MO. incharge ∞ Name of Supervisor Inspector & Designation POPULATION: Worker 6 Population 5 Name of village 4 Name of health Sub-3 Name of Panch-BLOCK: the 2 SL.

* Specific dates to be menioned.

ADVANCE SPRAY PROGRAMME

..... Total Population Targetted Population District

Name of M.O.	
Name of Inspector	
Name of Name of Name supervisor Inspector of M.O BHI	
Name of Health worker	
Name / No. of squad	
Dates for Spray	·
Popul- ation	
Name of Popul-Village ation	
Name of Panchayat / Village Sub centre	
o N S	

Note: To be circulated to all concerned
Requirement of insecticides & Spray
squads should be worked out in format separately



KALA-AZAR TREATMENT CARD

* (1.To be used only for patients not responding to full course of treatment of 20 inj. / Slow Responders / Relapsed case) (2. Not to be used at sub-centres or institutions where diagnostic & monitoring facilities not available.)

Dist	rićt:	Name of	Name of Institution			Name of Treatment Centre			
		of diagno	osis & Regd. No		& KA Reg.	No			
	ME								
Add	Iress Vill	. Panchayat	P.S.	Dist.	Occup	oation			
			ILS OF PREVIOU						
	Name of treatment centre								
	(A Regd. No								
Tre	atment complete / Inc	complete / Irregul	ar Slov	Responder / No	n - Responder / F	Relapsed			
Rel	evant finding on comp	oletion of treatmen	nt	••••••••••					
Rea	asons for referring for	2nd course / Rer	narks						
Dia	gnosis	EXA	MINATIONS (With	Dates)					
(1)	Clinical:	Date	II Date	 Data	IV Date	Remark			
	a) weight b) Loss of appetite c) Fever d) Anaemia e) Splenomegaly f) Hepatomegaly g) Any other	Date	Date	Date	Date				
(2)	Lab.: a) TLC b) DLC c) Hb d) Urine: Sugar: Alb e) Bone Marrow f) Splenic Puncture								
(3)	Any other : a) b)								
Pres	2. 3. 4. 5.	ml deep l.m. signation & signatu	daily for o	days					

मुख्य सन्देश

- कालाजार लाइलाज नहीं है, समय पर समुचित इलाज से रोगी पूर्णतः रोग मुक्त हो जाता है।
- बुखार ठीक होने पर भी बीच में सुई लगवाना बन्द नहीं करें और इलाज पुर्ण कराकर रोग मुक्त हों।
- कालाजार से बचने के लिए साल में दो बार डी० डी० टी० का छिडकाव जरूर करायें।
- अपने घर, गोहाल तथा आस-पास सफाई रखें।
- * To be used by MO Incharge PHC / Higher Levels, to be printed preferably in red colour

SSG course details

Injection No.	Date	Injected by (Signature)	Patient's Signature	MO's Remarks with Signature	Injection No.	Date	Injected By (Signature)	Patient's Signature	MO's Remarks with Signature
*1					**11				
2.					12.				
3.					13.				
4.					14.				
5.					15.				
6.					16.				
7.					17				
8.					18.				
9.					19.				
10					20.				

^{*} Treatment will be given to patient who have already received a full course of 20 injections. Injection no.1 means 21 st injection for the patient. (20 injections on Green Card)

Treatment started on :
Completed on
Cured / Referred to
Remarks

^{** 11}th and subsequent injection should be provided under strict Medical Supervision.

KALA-AZAR TREATMENT CARD

CARD No :

** (To be used for patients receiving treatment for the first time)

District : Name of Institution						atment center
		of diagnos	is & Regd. No		& KA Reg. No	
NAN	ΛΕ	S/	o D/o W/o		Age	Sex
Address Vill.		Panchayat	P.S	Dist	Occupa	ation
		AMINATIONS (EV				
(1)	Clinical:	Date	II Date	III Date	IV Date	Remark
	a) weight b) Loss of appetite c) Fever d) Anaemia e) Splenomegaly f) Hepatomegaly g) Any other					
(2)	Lab.: a) TLC b) DLC c) Hb d) Urine Sugar Alb e) Bone Marrow f) Splenic Puncture					
(3)	a) b)		de il ofere 200 de un	1		
	2 3 4 5	ml deep l.m. designation & signat				

मुख्य सन्देश

- कालाजार लाइलाज नहीं है, समय पर समुचित इलाज से रोगी पूर्णतः रोग मुक्त हो जाता है।
- बुखार ठीक होने पर भी बीच में सुई लगवाना वन्द नहीं करें और इलाज पुर्ण कराकर रोग मुक्त हों।
- 3. कालाजार से बचने के लिए साल में दो वार डी० डी० टी० का छिड़काव जरूर करायें।
- x अपने घर, गोहाल तथा आस-पास सफाई रखें।

(Note ** To be used for first course by PHC / Additional PHC. To be printed preferably in green colour)

SSG course details

Injection No.	Date	Injected by (Signature)	Patient's Signature	MO's Remarks with Signature	Injection No.	Date	Injected By (Signature)	Patient's Signature	MO's Remarks with Signature
*1					**11				
2.					12.				
3.					13.		·		
4.					14.				
5.					15.				
6.					16.				
7.					17.				
8.			,		18.				
9.					19.				
10.					20.				

Report from treatment Centre

Treatment Started on :	Troport from troumont control	•"
Completed on		
Full Course Treatment	Treatment started on :	
Tull Course Treatment	Completed on :	*****
Completed / Incomplete	Cured / Referred to	
Patient Cured		
Referred for Re-exam.	Remarks	
Any other		

SAMPLE ADVANCE INTIMATION CARD (Used in Bihar)

डी० डी० टी० का छिड़काव करायें। कालाजार दूर भगायें



अग्रिम छिड़काव सूचना कार्ड

गाँव का नाम
छिड़काव की तिथि
पर्यवेक्षक का नाम
चिकित्सा पदाधिकारी का नाम

कालाजार से बचाव के लिए डी० डी० टी० का छिड़काव आवश्यक है। अपने घरों के पूर्ण छिड़काव में अपना सक्रीय सहयोग दें।

जानने योग्य बातें

- छिड़काव से पुर्व अपने घरों एवं गोहालों की सफाई करें।
- दीवाल के सभी गड्ढों एवं दरारो को भर दें।
- कमरे के सभी सामानों को कमरे के मध्य में रखकर
 ढक दें।
- घर के सभी कमरों, गोहाल, रसोई—घर एवं पूजाघर
 में छिड़काव अवश्य करायें।
- रसोई-घर अथवा पूजाघर में छिड़काव में परेशानी हो तो स्वास्थ्य कार्यकर्त्ता की मदद से आप स्वयं छिडकाव कर लें।
- छिड़काव के पश्चात ढाई—तीन महीने तक दीवाल की लिपाई—पुताई नहीं करें। डी० डी० टी० का प्रभाव जाता रहेगा।

सन्देश

- कालाजार लाइलाज नहीं है, समय पर समुचित इलाज से यह रोग पूर्णतः ठीक हो सकता है।
- बुखार ठीक होने पर भी बीच में सूई लगवाना बन्द नहीं करें,
 कम—से—कम २० दिन सूई लगातार अवश्य लगवाएँ।
- ३. अपने घर, गोहाल एवं आस—पास सफाई रखें, कालाजार का प्रसार अपने आप कम हो जाएगा।
- ४. कालाजार से बचने के लिए साल में दो बार डी० डी० टी० का छिड़काव जरूर करायें।

Key to Species Identification*

The key basically covers 46 species recorded from India. however the presently known species from Bangladesh and Nepal could be separated with the help of this key.

Key to Genera

(1) Hairs of abdominal tergites 2-6, uniformly erect, cibarium without teeth, if present, only in the from of minute spicules and not in a definite row. Pigment patch always absent.

- Phlebotomus

- Hairs of abdominal tergites 2-6 recumbent or with a few erect hairs, cibarium teeth and denticles usually in transverse rows present. Pigment patch always present.

- Sergentomyia

Key to Subgenera of genus *Phlebotomus*Males

(Single species under subgenera are indicated).

- Cibarium with teeth. Pharynx unarmed. Palps not extending further than antenna 3.

- Subgenus Idiophlebotomus

- Cibarium with spicules or unarmed. Pharynx usually armed. Palps extending further than antenna 3. - 2

^{*} Source SEA / IBC / 35. WHO SEARO.

(2) Terminalia very long, Paramere with 2 long dorsal processes. Style long with 5 short spines. Coxite with long and thick setae in distal part and very small basal process. Surstyles with short spines.

- Subgenus Phlebotomus

- Terminalia short or middle size. Paramere without or with short ventral process. Style with long spines. Coxite without setae in distal part without or with large basal process. Surstyles without spines.

 3
- (3) Style with 4 spines.

- 4

- Style with 5 spines, rarely with 6 spines

- 5

- (4) Coxite with basal process. Paramere without ventral process.
 - Subgenus Paraphlebotomus
- Coxite without basal process, Paramere with a ventral process.
 - Subgenus Anaphlebotomus
- (5) Coxite with basal process

-Subgenus *Synphlebotomus* (Only one species *P.eleanorae*)

- Coxite without basal process.

- 6

- (6) Paramere with 1-2 ventral processes or a tubercle. Aedeagus short, with long lateral spine.
 - Subgenus Euphlebotomus

- Parameres without ventral processes rarely with tubercle.

 Aedeagus long, without lateral spine. 7
- (7) Genital filaments 6-11 times length of genital pump. Aedeagus with sub-terminal tooth.

- Subgenus Adlerius (Only one species P. longiductus)

- Genital filaments 3 - 3.5 times length of the genital pump. Aedeagus with sub-terminal tooth.

- Subgenus Larroussius (Only one species P.major major)

Females

(Single species under sub-genera are indicated)

(1) Cibarium with teeth. Pharynx unarmed. Palp not extending further than antenna 3.

- Subgenus Idiophlebotomus

- Cibarium with spicules or unarmed. Pharynx usually armed. Palp extending further than antenna 3. -2
- (2) Spermatheca with regular segmentation (rarely indistinct) or nearly round.
 - Spermatheca spindle-shaped, with irregular segmentation.

- Subgenus Adlerius (Only one species P. longiductus)

- (3) Head of spermatheca with distinct neck. 4
 - Head of spermatheca without neck. 5

(4) Head of spermatheca with long neck. Chitinous arch not developed. Cibraium almost unarmed.

- Subgenus Larroussius (Only one species *P.major major*)

- Head of spermatheca with short neck, Chitinous arch well developed. Cibarium with distinct horizontal teeth.

- Subgenus Anaphlebotomus

- (5) Apical segment of spermatheca enlarged or separated from other by more deep furrow. 6
 - Apical segment usual, not separated from other 7
- (6) Spermatheca with 2-9 segments.
 - Subgenus Paraphlebotomus
 - Spermatheca with more than 15 segments.
 - Subgenus **Euphlebotomus**
- (7) Spermatheca with wide head.
 - Subgenus Phlebotomus
 - Spermatheca with narrow head
 - Subgenus Synphlebotomus

Key to the species of subgenus Idiophlebotomus (Females)*

(1) Cibarial armature, with medium rod and cibarium teeth in radiating lines.

- P.tubifer (Recorded from India)

- Cibarial armature, without medium rod and cibarium teeth unequal, medium longest.

- P.teshi
(Recorded from Bangladesh)

(*Males of *P.tubifer* and *P.teshi* are still unknown)

Key to the species of subgenus *Phlebotomus* (Figs. 1, 2)

(1) Males
Females

(2) Distal part of parameres strongly curved up; second dorsal process of paramere rather short and widened at the end; surstyle with seven spines large to very small.

- P.salehi

- Distal part of parameres straight or slightly curved up. Second dorsal process long and thin, surstyle with two or three similar spines.

- P.papatasi

(3) Pharynx with uniform squamae, spermathecae cylindrical

- P.papatasi

- Squamae in the centre of pharyngeal armature more hard and pigmented; spermatheca narrowing to the base

- P.salehi

Key to species of subgenus *Paraphlebotomus* (Figs. 3, 4)

(1) Male - 2 Females - 3

(2) Style very short $(76-100\mu)$ with 2 terminal spines or sometime 1 terminal and 1 subterminal. Basal process with asymmetrical thin head, directed obliquely down.

- P.sergenti

- Style longer than 100μ , with 1 terminal and 1 subterminal spines. Basal process with thick mushroom shaped head and fan shaped long hairs.

- P.alexandri*

(3) Spermatheca 4-5 segmented, with global apical segment. Pharynx with uniform blunt teeth, directed obliquely down, to the centre.

- P.sergenti

- Spermatheca 6-9 segmented; apical segment rather narrow. Pharyngeal armature occupies only base of the pharynx and almost rectangular in shape.

- P.alexandri*

(* Likely to occur in India bordering Sind province of Pakistan.)

Key to species of subgenus *Anaphlebotomus* (Figs. 8, 11)

(1) Male - 2 Female - 3

(2) Parameres bilobed. - P.colabaensis
Paramere trilobed. - P.stantoni

(3) Spermatheca slightly carrot shaped with small end segment. Individual duct about four times length of spermatheca.

- P.colabaensis

- Spermatheca spindle shaped with narrow cylindrical apical segment. Individual duct slightly longer than spermatheca.

- P.stantoni

Key to species of subgenus *Euphlebotomus* (Figs. 7, 9)

- 2
Female
- 3

(2) Paramere with 2 ventral process. Paramere with long processes and long distal parts. Coxite wide.

- P.argentipes

- Paramere with 1 ventral process or with only ventral tubercle. Parameres with wide, truncated straight end. Halteres long triangular

- P.newsteadi

(3) Spermatheca long with about 30 segments.

- P.newsteadi

- Spermatheca short with about 15-17 segments

- P.argentipes

Key to subgenera of the genus Sergentomyia Males (Figs. 12, 14)

(1) Aedeagus thick, finger-shaped. Paramere with blunt or slightly hooked ends.

- Subgenus Sergentomyia

- Aedeagus gradually tapering to the end. Paramere with hooked ends (except *S. indica*). -2
- (2) Genital filaments with dilated ends Mesanepisternum with two groups of hairs. A3 without ascoid.

- Subgenus Grassomyia

(only one species S. Indica)

- Genital filaments with narrow ends. Mesanepisternum without hair. A3 with one ascoid.
- (3) Aedeagus with blunt ends.

-4

- Aedeagus with sharply pointed ends.
 - Subgenus Sintonius

(4) Paramere with hairy tubercle on ventral side. Genital filament with marked transverse striations.

- Subgenus Neophlebotomus

- Aedeagus slender, triangular and narrowing gradually to a sharp point. Paramere without tubercle. Genital filament with or without striations

- Subgenus Parrotomyia

Females (Figs. 12, 14)

(1) Spermatheca with regular segmentation.

- Subgenus Sintonius

- Spermatheca without regular segmentation. 2
- Spermatheca with capsule. 3
- Spermatheca without capsules and tubular. 4
- (3) Capsules of spermatheca with numerous spicules or striations. Antennal formula 1/4-15.

- Subgenus **Grassomyia**

- Capsule of spermatheca smooth.

- Subgenus **Parrotomyia** (only one species S. indica)

- (4) Spermatheca smooth Subgenus Sergentomyia
 - Spermatheca striated. Subgenus Neophlebotomus

Key to the species of Subgenus Sergentomyia (Fig. 12)

(1) Male - 2 Female - 3

(2) Parameres hooked. - S.punjabensis

Parameres blunt.

(3) Spermatheca tubular with smooth sides and wide duct, hind teeth of pharyngeal armature much smaller than fore teeth. Hind width of pharynx about 0.58-0.77 of length.

- S.punjabensis

- S.theodori

- Hind pharyngeal armature is not much smaller than fore teeth, length, of pharynx 2.26 or more times of hind width.

- S.theodori

Key to the Species of Subgenus Sintonius Males (Figs. 15, 16, 19, 22)

- (1) Cibarium with 2-15 teeth. Pale sandflies. 2
 - Cibarium with 16-35 teeth. Dark sandflies. 4
- (2) Cibarium with 2-5 widely spaced teeth and several vertical denticles, pigment patch small, drop shaped. A3 with one papilla.

- S. christophersi

- cibarium with 9-15 teeth.

- 3

(3) Antenna 3 = 0.06 length of wing
- S.sirohi
- Antenna 3 = 0.11 to 0.12 length of wing. Cibarial teeth on convex arc in centre.
- S.orissa
(4) Cibarial teeth minute and arranged in group.
- S.clydei - Cibarial teeth otherwise 5
(5) Cibarium with about 20 teeth.
- S.eadithae
- Cibarium with 28 teeth S.hospitii
Females (Figs. 15, 16, 22)
(1) Pharynx very heavily armed. Cibarium with about 27 teeth in pallisade-like convex curve. Two row of fore teeth present.
- S.orissa

- Pharynx lightly armed or unarmed.

- 2

(2) Pharynx with distinct spicules; cibarium with convex row of 70-80 long teeth and very wide almost black pigment patch.

- S.hospitii

- Pharynx with minute spicules or none.

- 3

	Cibarium with about 35 large pigmented teeth in a rovex medially
	- S.eadithae
	- Cibarium with 18 teeth or less 4
(4)	Cibarium with 5 widely spaced narrow teeth.
	- S.christophersi
	- Cibarium with 10-18 teeth close together 5
	- Cibarium without fore teeth S.sirohi
	- Cibarium with fore teeth S.clydei
	to the species of subgenus <i>Parrotomyia</i> es (Figs. 20, 21, 23, 25, 27, 29)
, ,	Tip of aedeagus pointed- style with four apical spines arium with no fore teeth but with 20-30 hind teeth.
	- S.africana magna
	- Tip of aedeagus rounded 2
(2)	Style with three large and one small spine.
	- S.himalayensis
	- Style with four equal spines 3

(3) Style about five or six times as long as thick. Antenna 3 about 0.16 to 0.18 mm long.
- S.babu babu - S.baghdadis - S.shorttii
- Style about 4 times as along as thick 4
(4) Two of spines on style sub-apical.
- S.sp(A)
- All spines on style apical5
(5) Cibarium with about 18 teeth.
- S.barraudi
- Cibarium with about 10-13 teeth.
- S.sp(B)
(*Males of S. modii and S. kauli are unknown. Males of sp(D) and (E) recorded from Bihar, but description not available.)
Females* (Figs. 20, 21, 23, 24, 25, 27, 29, 30)
(1) Pharynx with many distinct pointed teeth 2
- Pharynx with very fine spicules or none 7
(2) Cibarium with notch in hind end of ventral plate 3
- Cibarium without notch in hind end of ventral plate.

(3) Cibarium with about 10-14 teeth, notch shallow.

- S.shorttii

- Cibarium with 24-34 teeth, notch deep.

- S.babu babu

(4) Pharynx broad, with many long finely pointed teeth. Cibarium with 40-70 teeth and tip of pigment patch bifid ragged or fenestrated.

- S.barraudi

- Without this combination.

- 5

(5) Cibarium with 64 teeth or more; Pharyngeal teeth short, hind margin of pigment patch concave.

- S.himalayansis

- Cibarium with 60 teeth or less.

- 6

(6) Cibarium with 42-50 teeth.

- S.africana magna

- Cibarium with 26-32 teeth on distinct arch.

- S.kauli

(7) Cibarium with deep notch in hind end of ventral plate, Cibarium with 16-20 teeth; Pharynx with transverse ridges and with some hind spicules.

- S.baghdadis

- Cibarium without such notch. Cibarium with 17 teeth and a pigment patch.

- S.modii

(*Females of S.sp(A), S.sp(B), S.sp(D) and S.sp(E) are unknown.)

Key to the species of Subgenus Neophlebotomus Males* (Figs. 26, 28, 31, 32, 33, 34, 35, 36)

- (1) Style with two of spines near middle. 2
 - Style with spines terminal or sub-terminal. 5
- (2) Style with seta at about 0.8 coxite with long brush with 60 hairs.

- S.purii

- Style with seta at 0.7 or more proximal. 3
- (3) Coxite long, narrow and slightly curved.

- S.perturbans

- Coxite otherwise.

- 4

(4) Aedeagus thick, mid-width of shaft about 0.19 of extreme length of aedeagus, coxite with some of outer hairs concentrated.

- S.zeylanica

- Aedeagus slender, mid-width of shaft about 0.11 of extreme length of aedeagus. Cibarial fore teeth not in broad band. Brush starting at 0.21 of coxite.

- S.arboris

- Two spines of style at 0.76-0.85; cibarium with ten scattered hind teeth and no fore teeth.

- S.malabarica

(6) Paramere with spinose process at base of neck.

- S.hodgsoni hodgsoni

- Paramere without such process.

- 7
- (7) Cibarium with long narrow pigment patch.

- S.linearis

- Cibarial pigment patch not long and narrow. 8
- (8) Cibarium with conspicuous fore teeth.

- S.iyengari

- Cibarium without fore teeth.

- S.dhandai

(*Male of S. chakravarti unknown.)

Keys to the species of Subgenus *Neophlebotomus* Females (Fig. 26, 28, 31, 32, 34, 36)

(1) Cibarium with about eight rows of fore teeth.

- S.arboris

- Cibarium with less than eight rows of fore teeth. -2

(2) Cibarium with three rows of fore teeth. Fore teeth of hind rows not very large. - S.zeylanica - Cibarium with less than three rows of fore teeth or none. - Cibarial central teeth marked by different from rest. (3) - 4 - Cibarial teeth not marked by different from rest. (4) Cibarium with 24 teeth - S.dhandai - Cibarium with 14-17 teeth. - S.iyengari (5) Cibarial pigment very narrow and linear - S.linearis - Cibarial pigment patch not very narrow. - 6 Cibarium with 50-60 teeth. (6)- S.hodgsoni hodgsoni - Cibarium with about 20 teeth or less. (7) Cibarium with about 20 teeth in concave row. - S.purii

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- Cibarium with less than 20 teeth on average. - 8

(8) Cibarium with 8 or 9 main hind teeth arising from refractive brown area with colour different from pigment patch.

- S.perturbans

- Cibarium otherwise.

- 9

(9) Cibarium with 14 hind teeth in a line angular at the centre, 7 round teeth present behind hind ones

- S.chakravarti

-Cibarium with about 8 hind teeth in nearly straight row. No teeth behind hind ones

- S.malabarica

Nicnic and ungrouped (Males and Females)* (Figs. 17, 18)

- Cibarium with several rows of lateral teeth; Pigment patch with broad process and narrow hind part. Pharynx narrow with many teeth.

- S.montana

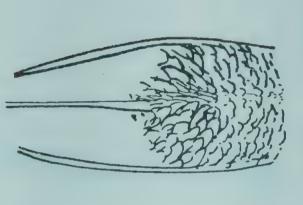
- Cibarium of females with one or more rows of small, sometimes scarcely visible hind teeth. Spermatheca smooth, on elliptical or cylindrical capsule; Pharynx of females without spicules; Aedeagus pointed and parameres hooked, style with all species terminal or two of them sub terminal.

- S.bailyi

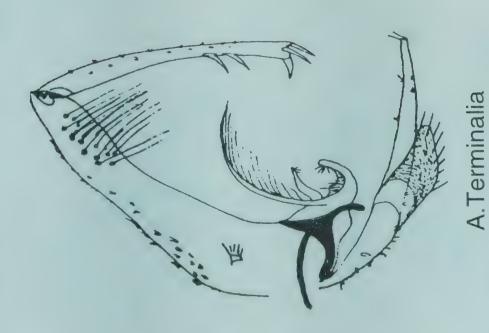
(Males of S.sp(C) & S.sp(F) are recorded from Bihar (Kaul et al, 1979). Description of these species not available. Females are unknown.)

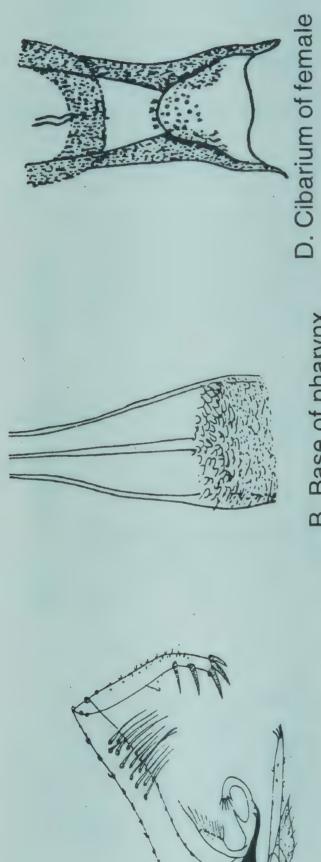


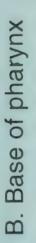
C. Spermatheca



B. Base of pharynx



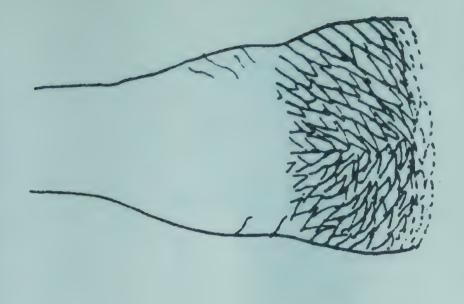




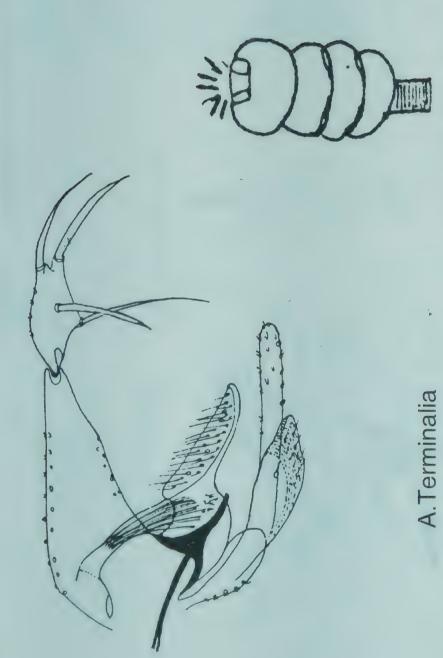


C. Spermatheca

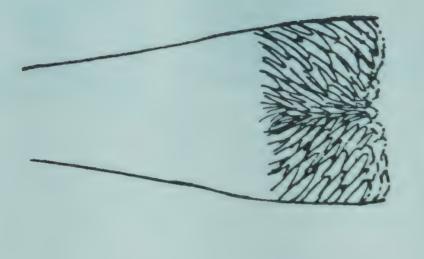
A. Terminalia



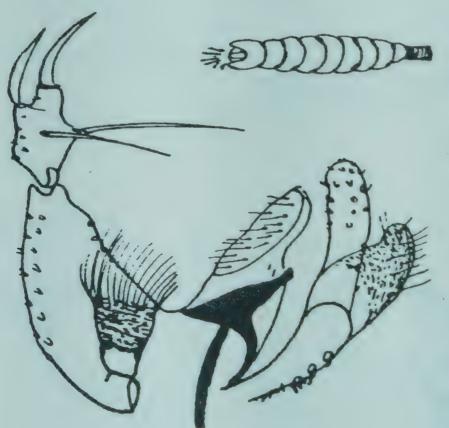
C. Base of pharynx



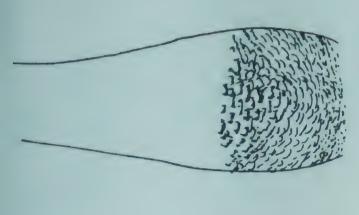
B. Spermatheca



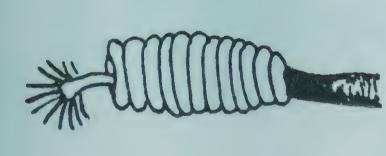
C. Base of pharynx



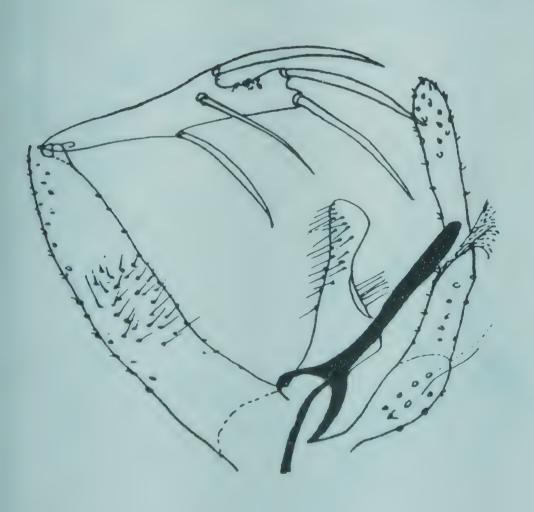
A. Terminalia B. Spermatheca



C. Base of pharynx



B. Spermatheca



A. Terminalia

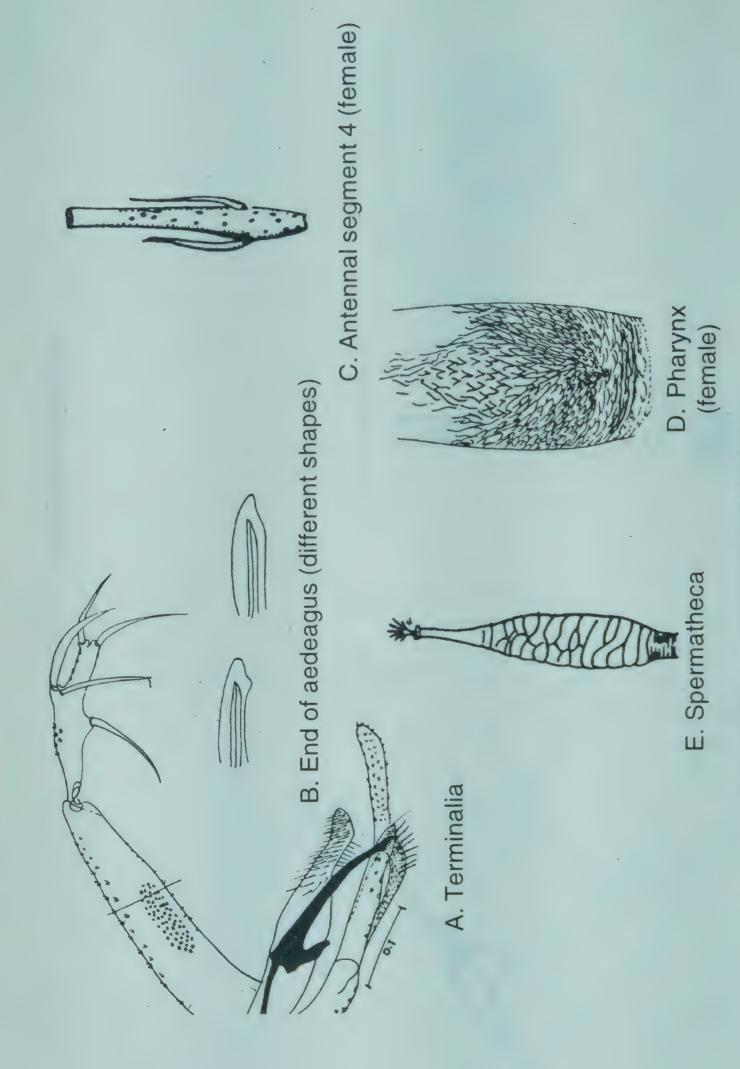
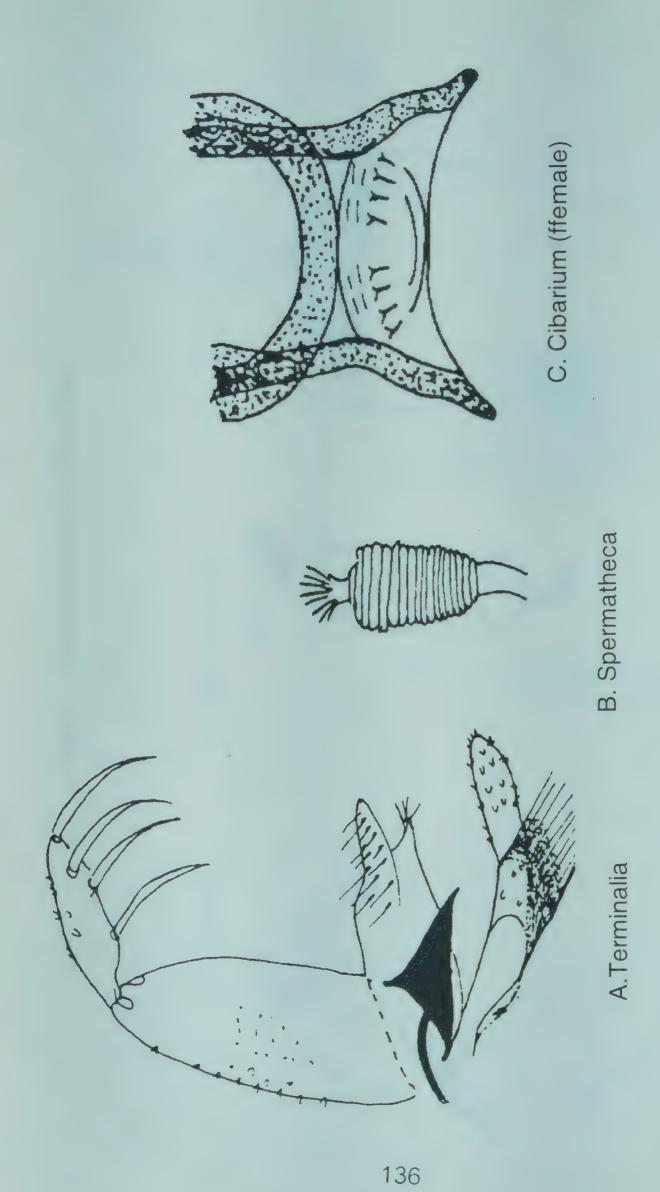
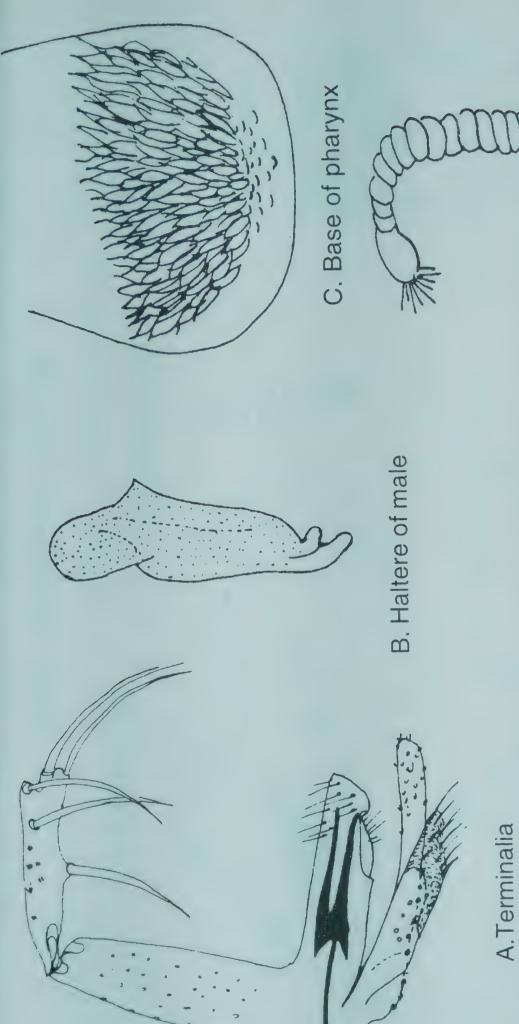


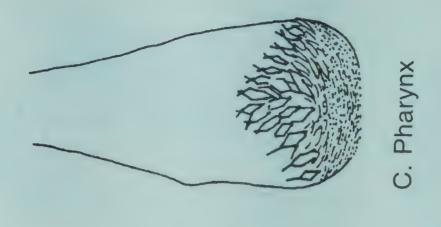
Fig. 6 Sergentomyia longiductus

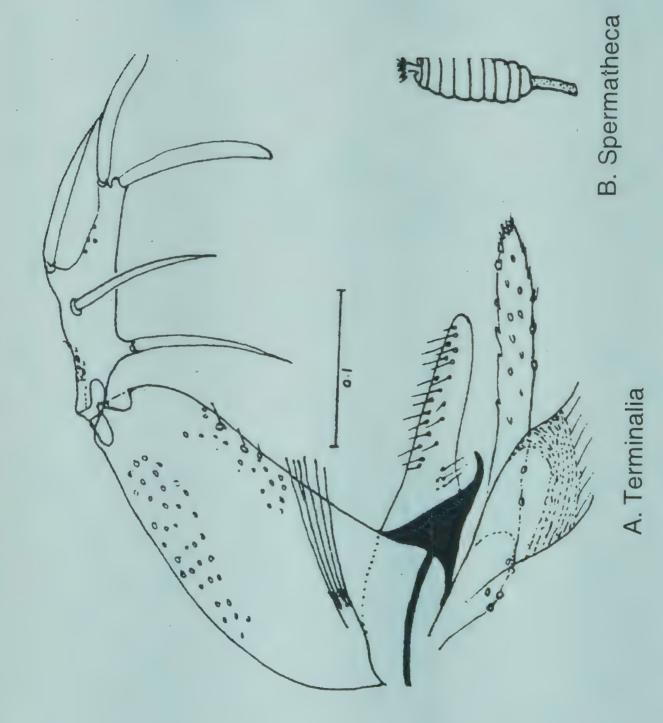
Fig. 7 Phlebotomus argentipes



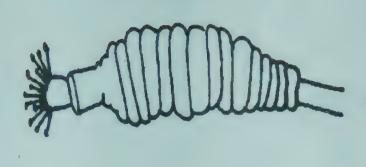


D. Spermatheca





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C. Spermatheca capsule



B. Spermatheca with ducts

A. Cibarium

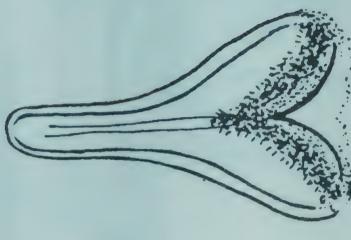
Fig. 11 Phlebotomus stantoni

Fig. 12 Sergentomyia punjabensis

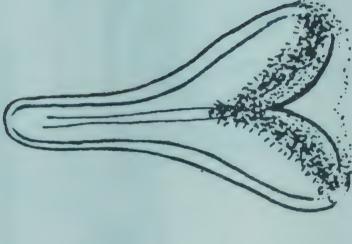
E. Labrum (male)

D. Pharynx (male)

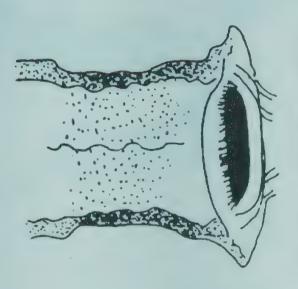




H. Pharynx (female)



G. Cibarium (female)



F. Head (female)

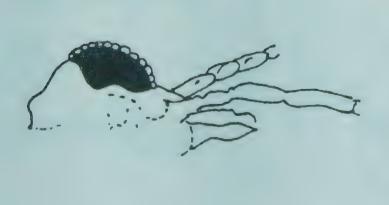
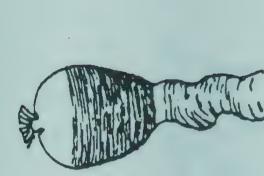


Fig. 12 Sergentomyia punjaben'sis

C. Cibarium (male)

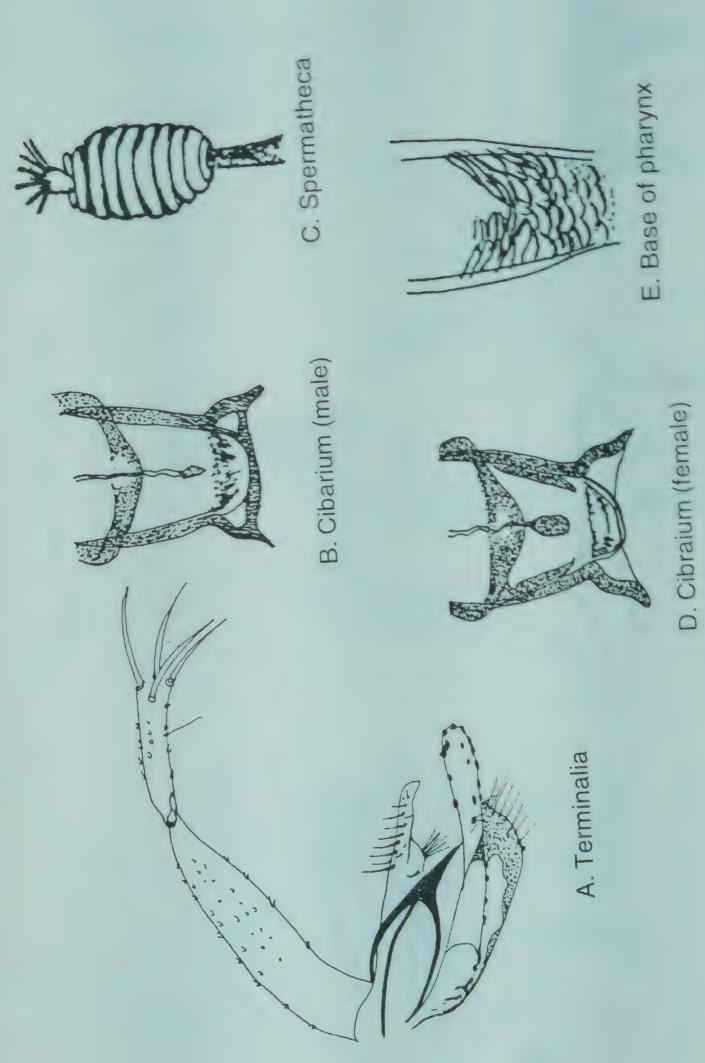
Fig.13 Sergentomyia hodgsoni hodgsoni



D. Spermatheca

A. Terminalia

Fig.14 Sergentomyia indica



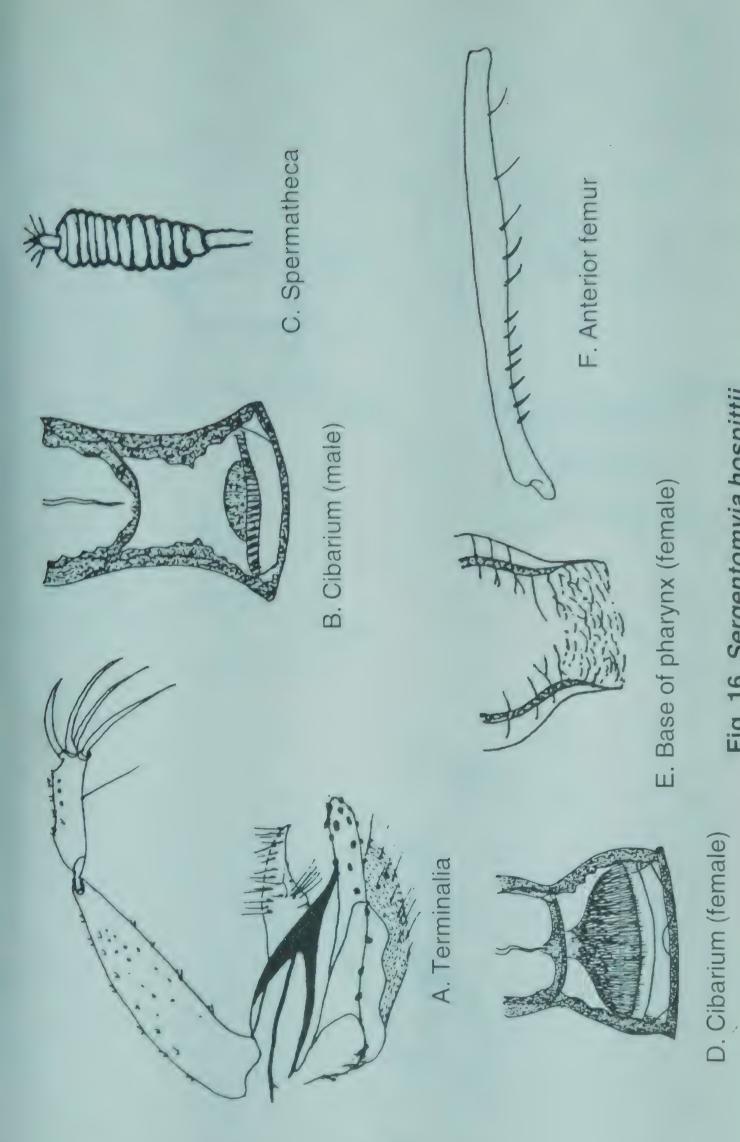
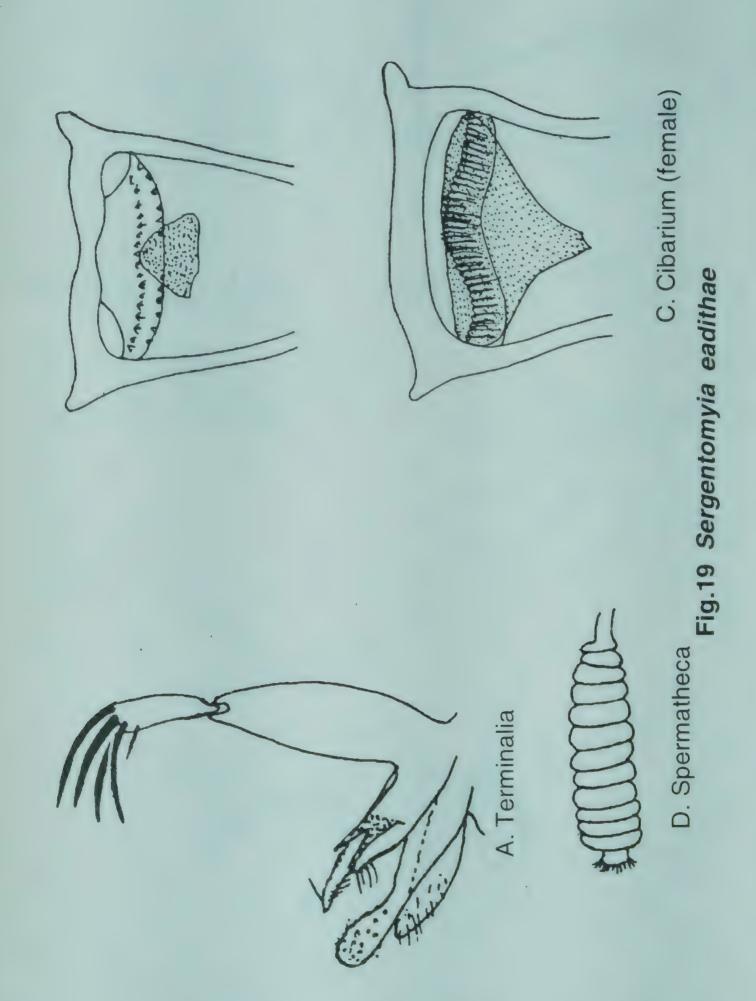
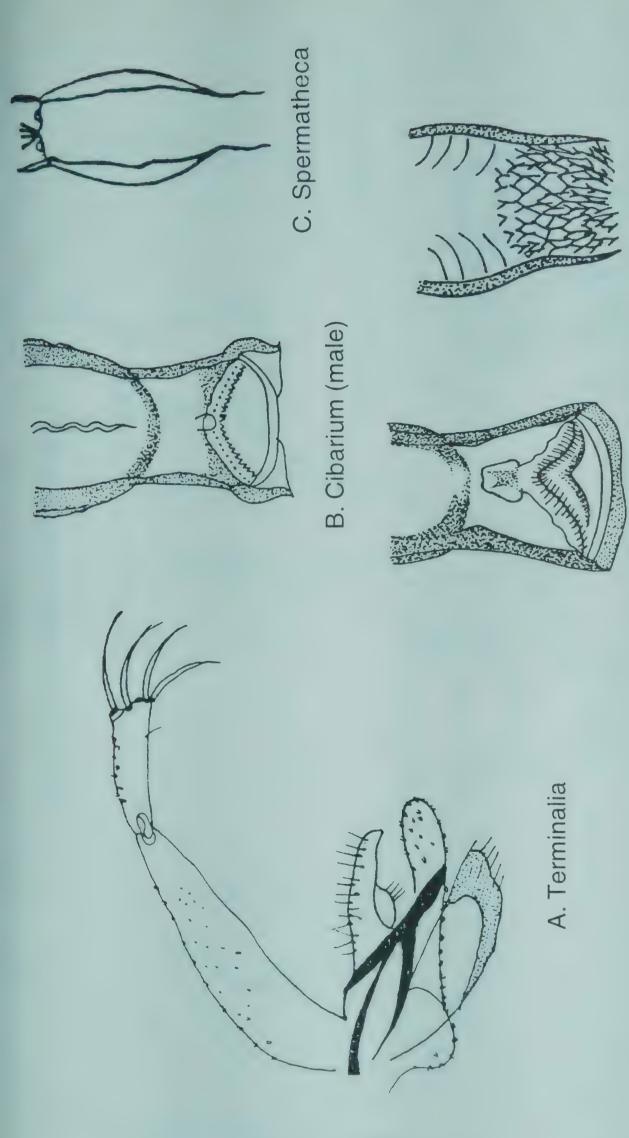


Fig. 16 Sergentomyia hospittii

Fig.17 Sergentomyia montana

Fig. 18 Sergentomyia bailyi





Base of pharynx (female)

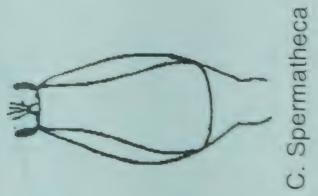
D. Cibarium (female)



B. Cibarium (Female)



D. Base of pharynx (female)



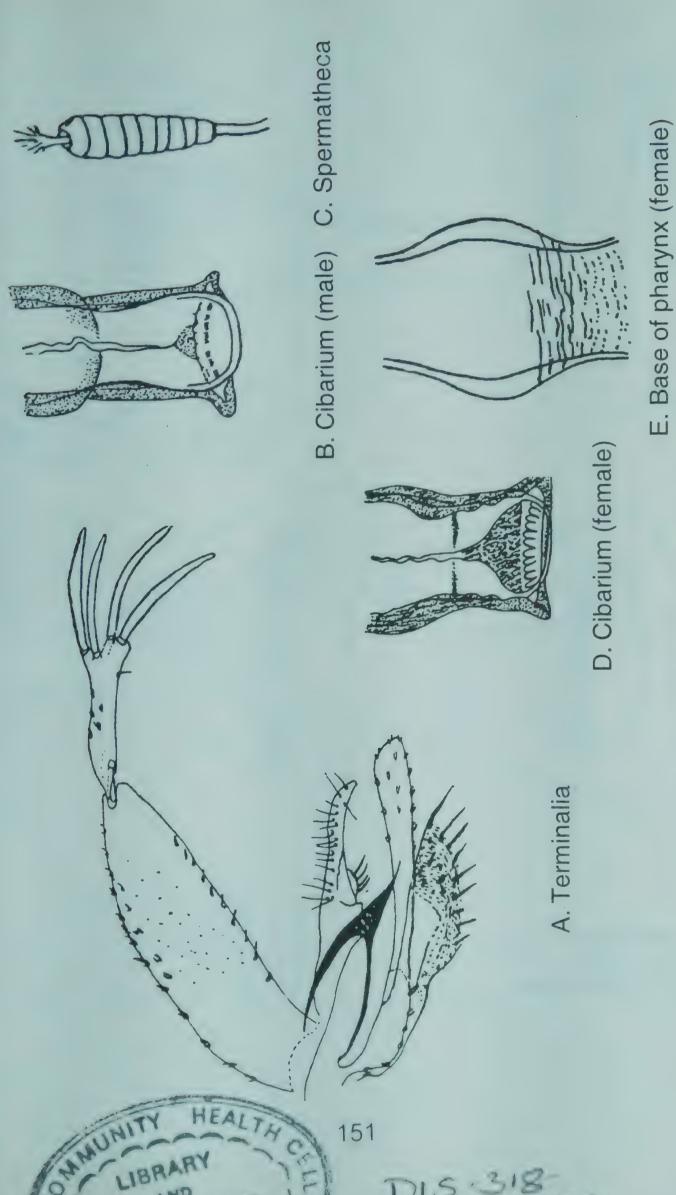
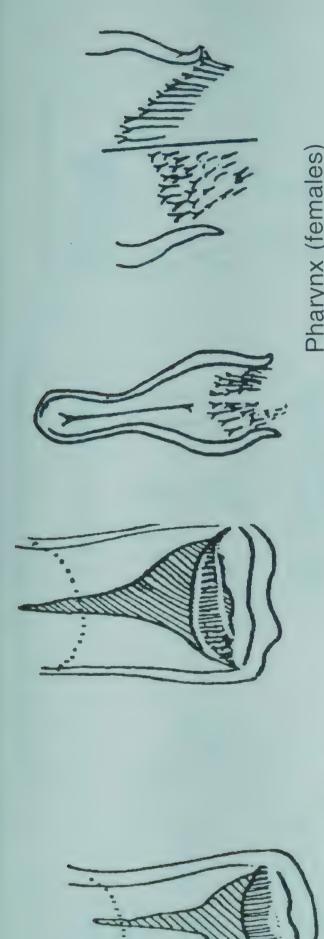


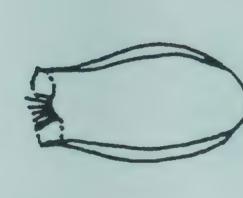
Fig.22 Sergentomyia clydei

Fig.23 Sergentomyia africana megna

A. Style



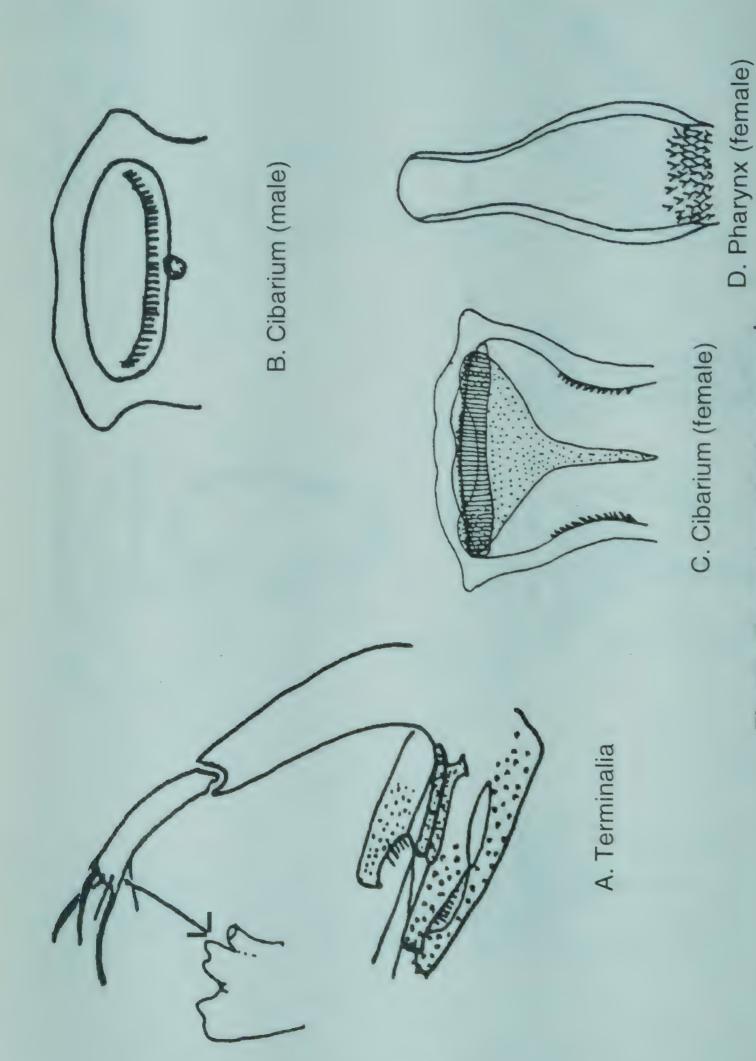
Pharynx (females)



Spermatheca

Fig. 24 Sergentomyia kauli

Cibarium (female)



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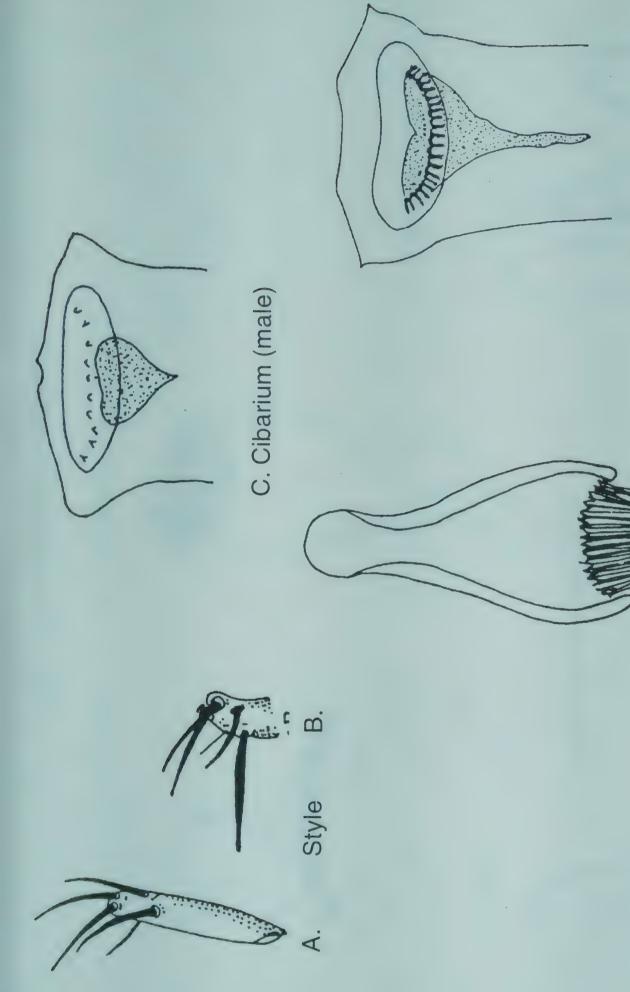
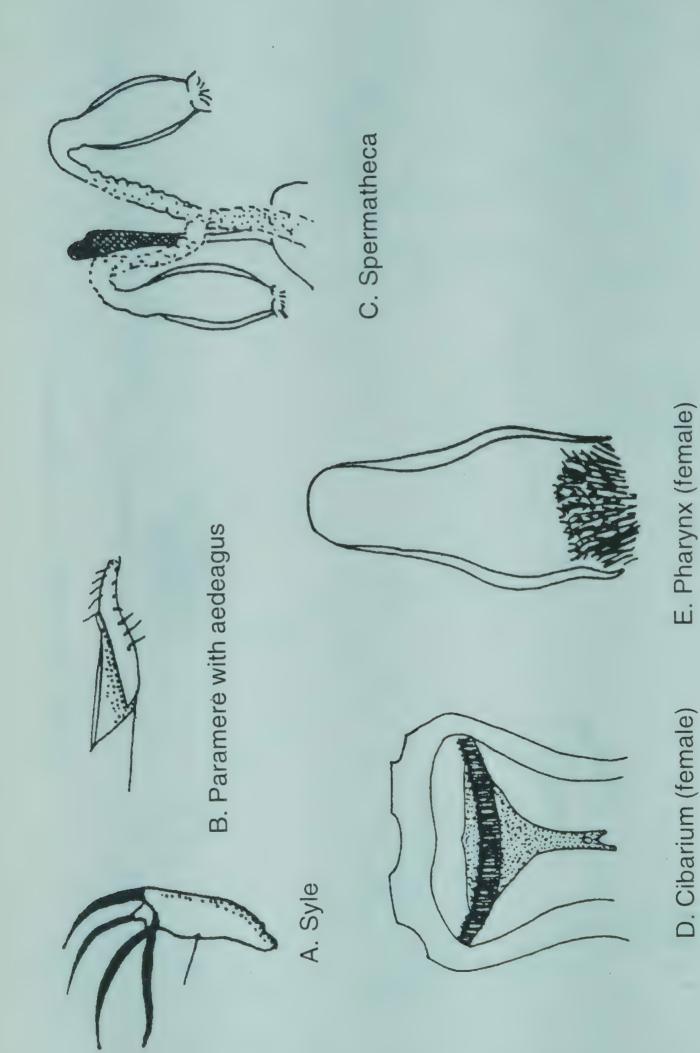
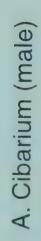


Fig. 26 Sergentomyia malabarica

E. Pharynx (female)

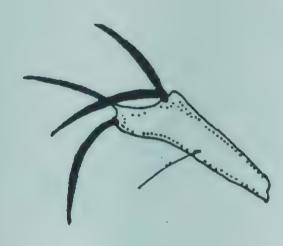
D. Cibarium (female)







B. Paramere with aedeagus

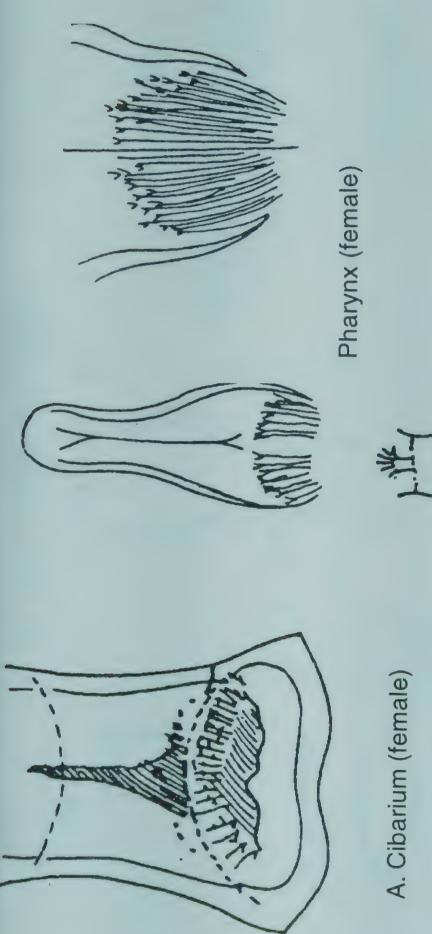


C. Style

Fig.28 Sergentomyia arboris



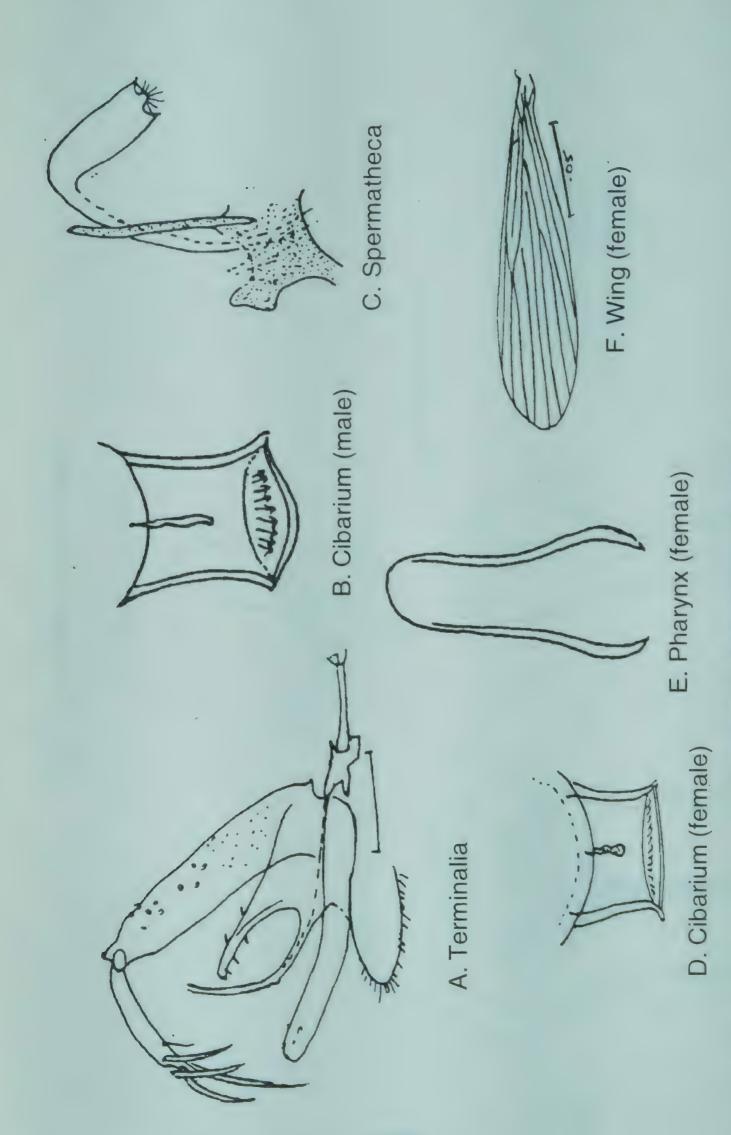
A. Cibarium (female)

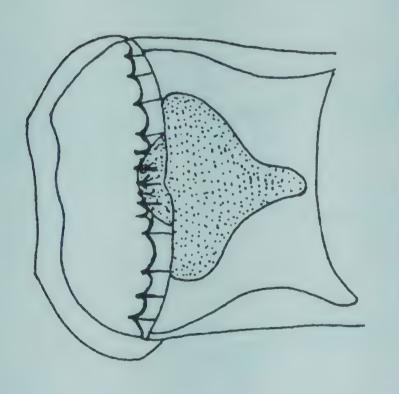




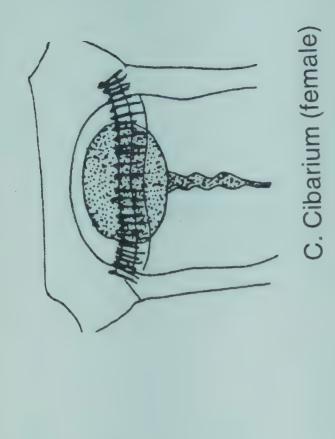
D. Spermatheca

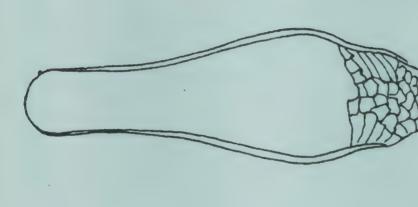
Fig.30 Sergentomyia modii





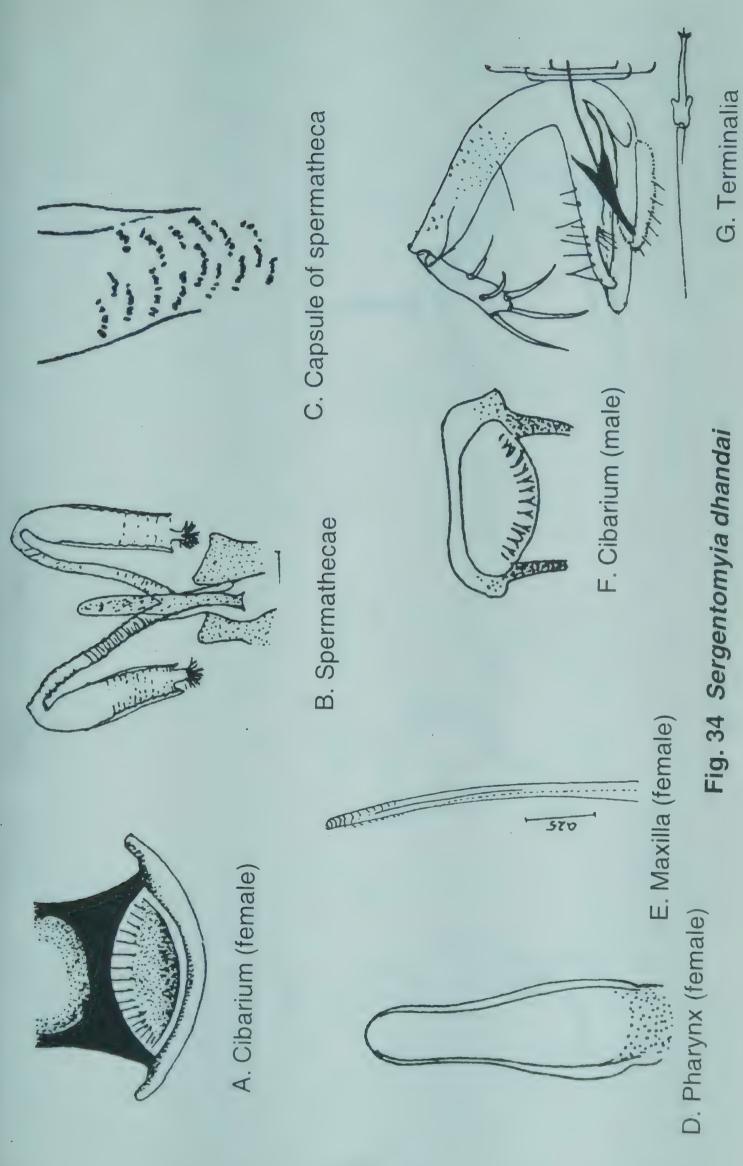
A. Cibarium (female)

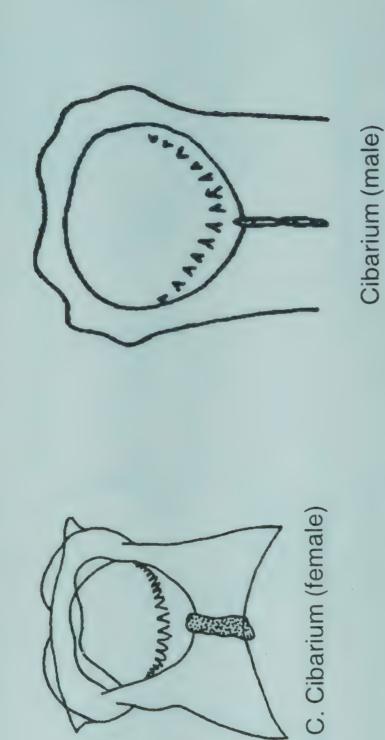




B. Pharynx

A. Terminalia (Style)





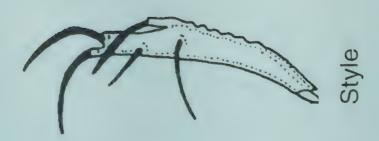
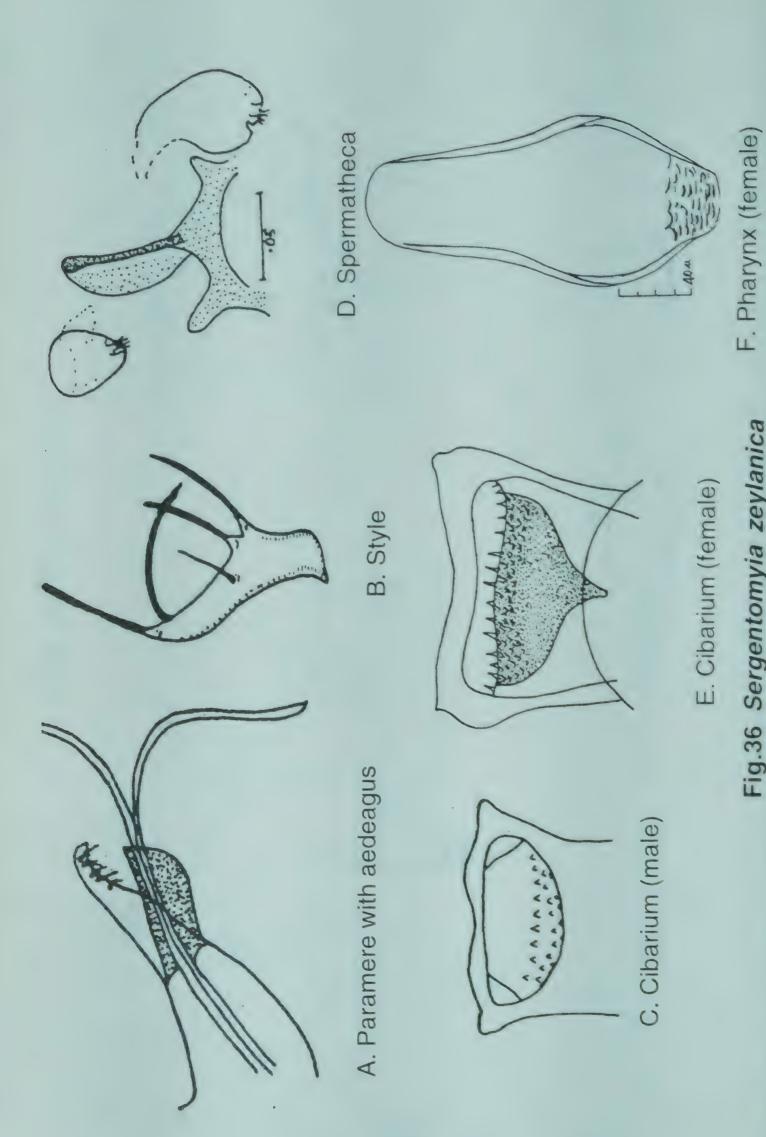


Fig.35 Sergentomyia purii

Spermatheca



STATEWISE KALA - AZAR CASES AND DEATHS IN INDIA (1977 - 1980)

SI.	State	С	1977 D	С	1978 D	С	1979 D	C	1980 D
						6	1	-	
1.	Assam	10500	229	41980	62	25472	28	13620	25
2.	Bihar	18589	229	41300	- 02	2	-	-	_
3.	Delhi	8	_				_	-	-
4.	Jammu & Kash	nmir 1	-	~	-	,	_	_	
5.	Mahalaya	7	-	~	-	_			
		(Old)							_
6.	Pondichery	• 4	-	, •	~	-	-	_	
7.	Tamil Nadu	62	-	53	-	- ·	-	~	_
8.	Uttar Pradesh	9	-	-	-	-	- 4	222	-
9.	West Bengal	63		-	-	71	1	333	6
-	Total	18743	229	42033	62	25551	30	13953	31

STATEWISE KALA - AZAR CASES AND DEATHS IN INDIA (1981 - 1984)

SI. No.	State	С	1981 D	С	1982 D	С	1983 D	С	1984 D
1.	Assam	1	-	4	-		-	-	-
2.	Bihar	14165	36	11120	35	11832	128	12985	67
3.	Delhi	3*	_	1*	-	2*	-	1*	-
4.	Jammu & Kash	nmir -		1	-	-	**	•	***
5.	Tamil Nadu	2	_	-	-	~	-	2	-
6.	Uttar Pradesh	**	-	***			-	3	-
7.	West Bengal	917	5	1234	3	2717	7	4233	-
	Total	15088	41	12360	38	14551	135	17224	67

C = Cases : D = Deaths

STATEWISE KALA - AZAR CASES AND DEATHS IN INDIA (1985 - 1987)

SI. No.	State	С	1985 D	С	1986 D	С	1987 D
1.	Assam	-	**	4	~	5	2
2.	Bihar	13029	39	14079	47	19179	77
3.	Delhi	1*	-	4*	-	1*	-
4.	Mahalaya	-	-	1*	-	1 *	-
5.	Tamil Nadu	99	-	-	-	1*	-
6.	Uttar Pradesh	40	-	-	-	51	5
7.	West Bengal	4247	5	3718	25	4447	10+
	Total	17277	44	17806	72	23685	94

STATEWISE KALA - AZAR CASES AND DEATHS IN INDIA (1988 - 1991)

SI. No.	State	. C	1988 D	С	1989 D	С	1990 D	С	1991 D
1.	Assam	4*	-	3*	_	2*	_	-	
2.	Bihar	19639	123	30903	477	54650	589	59614	834
3.	Delhi	-	-	-		27*	-		-
4.	Karnataka		-	-	_	1*	-	-	-
5.	Maharashtra	9*	•	44	-	7*	-	1 *	-
6.	Tamil Nadu	19	_	4 4	-	10*	-	1 4	
7.	Uttar Pradesh	19	6	2	_	8		24	1
8.	West Bengal	3068	2+	3573	20	3037	.6	2030	3
	Total	22739	131	34489	497	57742	606	61670	838

C = Cases : D = Deaths

* Imported

STATEWISE KALA - AZAR CASES AND DEATHS IN INDIA (1992 - 1995)

SI.	State		1992		1993		1994	1995(F	Prov.)
No.		С	D	С	D	С	D	С	D
1.	Assam	1	w	2*	1*	and .	-	-	-
2.	Andhra Prades	h 1*	en .	~	~	eto	-	-	, 644
3.	Bihar	75523	1417	44155	706	24391	379	21045	259
4	Delhi	dar	400	-	-	55*	1*	13*	1 -
5.	Karnataka	/ **	-	1*	-	-	-	-	-
6.	Maharashtra	1	~				-		-
7	Uttar Pradesh	2*		3**	ALC:	57	1	15	-
8.	West Bengal	1574	2	1298	3	1149	3	1552	18
	Total	77102	1419	45459	710	25652	384	22625	277

C = Cases; D = Deaths

Note:- 1) * = Imported

2) ** = One case Imported

3) + = 19 Deaths for 1987 & 3 Deaths for 1988 were suspected due to Kala - Azar

4) - = Nil

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1977 - 1980)

ST	ATE : BI	HAR			C	= Cases	: D =	Deaths	
SI. No.	Affected District	С	1977 D	С	1978 D	С	1979 D	C	1980 D
1.	Patna	42	9	62	1	52	-	178	4
2.	Nalanda	-	_	15	_	-	-	8	
3.	Gaya	5		50	-	46	_	10	_
4.	Jahanabad *					, 0			
5.	Nawadah		_	_	-		-	-	-
6.	Aurangabad	1	-				-	1	
7.	Bhojpur	2	_	6	_	1	-	_	
8.	Rohtas	-	_	_	_				_
9.	Saran	89	_	363	_	476		282	_
10.	Siwan	27		268		535	-	88	
11.	Goopalganj	-	_	160	_	148	1	226	_
12.	East Champar	ran 218	_	2549	1	3659	2	1630	_
13.	West Champa		_	72	1	29	-	14	
14.	Muzaffarpur	5950	2	9373	24	4625	12	2242	1
15.	Sitamarhi	164	2	268	-	228		191	_
16.	Vaishali	6337	182	5885	8	4249	-	1522	
17.	Darbhanga	7	1	191	-	273	-	428	_
18.	Madhubani	10		24	_	94		75	
19.	Samastipur	3109	5	6794	6	2388	_	1159	_
20.	Bhagalpur	316	-	2566	_	1148	-	765	-
21.	Monghyr	344	-	1850	4	2077	-	788	2
22.	Khagaria(83)								
23.	Begusarai	577	11	1062	4	1145	-	568	2
24.	Purnea	34	-	784	5	1042	6	1212	5
25.	Katihar	580	1	869	-	898	-	1044	5
26.	Saharsa	455	16	4746	7	2358	7	1186	6
27.	Madhepura(86								
28.	Dumka	3	-	6	-		_	p4.	-
29.	Sahebganj(86								
30.	Ranchi		-	2	-	-	-		
31.	Hazaribag	62	_	2	-	-	-	-	-
32.	Giridih	22	-	-	-	-	-	3	
33.	Dhanbad	3	~	13	1	1	-		-
34.	Gooda			-	-		-	-	-
	Total	18589	229	41980	62	25472	28	13620	25

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1981 - 1984)

ST	ATE : BIH	IAR				C = Cas	ses ; D	= Deat	hs
SI. No.	Affected District	С	1981 D	С	1982 D	С	1983 D	С	1984 D
1.	Patna	191	2	219	-	269	10	361	7
2.	Nalanda	1	•	1	-	7	-	31	1
3.	Gaya	4	-	5	~	3	-	1	- 1
4.	Jahanabad *								
5.	Nawadah	•	-	1	~	-	-	-	-
6.	Aurangabad		-	1	-	~	-	-	-
7.	Bhojpur	de	-	-	-		-	4	-
8.	Rohtas	-	100		-	-	-	-	-
9.	Saran	216	~	510	-	469	-	406	1
10.	Siwan	108	~	119	-	19	-	61	-
111.	Goopalganj	247	2	180	-	125	-	48	-
12.	East Champar	an 829	940	205	-	225	-	271	-
13	West Champai	ran -		4	-	6	-	19	-
14.	Muzaffarpur	1126	2	338	1	257	-	428	3
15.	Sitamarhi	195	-	216	**	438	61	992	23
16.	Vaishali	716	, 100	351		282	8	242	1
17.	Darbhanga	224	-	676	2	804	-	476	2
18.	Madhubani	616	-	276	ass	509	2	368	1
19.	Samastipur	1124	-	574	~	569	13	485	5
20.	Bhagalpur	324	~	336	~	31	-	17	-
21	Monghyr	575	-	342	-	9	•	•	-
22.	Khagaria(83)					112	-	92	-
23.	Begusarai	312	-	152	~	120	-	241	-
24.	Purnea	5621	25	5117	15	5587	23	5937	20
25.	Katihar	973	2	761	3	1423	2	1615	3
26.	Saharsa	760	3	696	14	481	7	361	-
27.	Madhepura								
28.	Dumka	-	-	-	-	146	2	529	-
29.	Sahebganj								
30.	Ranchi	-	-	-	-	8	-	64	-
31.	Hazaribag	-		-	_	1	-	-	-
32.	Giridih	2	-	-	-	-	-	-	-
33.	Dhanbad	1	-	-	-	-	-	-	-
	Total	14165	36	11120	35	11832	128	12985	67

^{*} District created in 1987

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1985 - 1987)

SI	TATE : BIHA	AR			C = 0	Cases ; l	D = Deaths
SI. No.	Affected District	С	1985 D	С	1986 D	С	1987 D
1.	Patna	376	6	220	1	361	14
2.	Nalanda	32	-	35	-	30	-
3.	Gaya	-	-	-			-
4.	Jahanabad (87)					18	***
5.	Nawadah	-	-	-	-	q) to	-
6.	Aurangabad	-	-	-	-	-	-
7.	Bhojpur	52	6	11	-	44	-
8.	Rohtas	-	′ –	-	-	-	-
9.	Saran	548	-	463	-	341	-
10.	Siwan	325	-	306		163	-
11.	Goopalganj	125	-	107	-	95	-
12.	East Champaran	223	-	135		220	-
13.	West Champaran	24	-	22	. 1	28	1
14.	Muzaffarpur	542	2	1146	-	1302	8
15.	Sitamarhi	1450	4	1284	. 8	1030	11
16.	Vaishali	208	-	175	-	290	1
17.	Darbhanga	537	5	711	13	1123	10
18.	Madhubani	591	2	580	12	718	7
19.	Samastipur	726	1	1295	4	1400	19
20.	Bhagalpur	85	-	75	-	106	-
21.	Monghyr	-	-		-	-	-
22.	Khagaria(83)	183	-	67	-	84	-
23.	Begusarai	214	-	134	-	195	-
24.	Purnea	4263	13	3137	4	2781	3
25.	Katihar	1243	-	1038	2	897	1
26.	Saharsa	662	-	1368	2	1024	2
27.	Madhepura			403		-	-
28.	Dumka-	660	-	21	~	11	-
29.	Sahebganj	•	-	1346	-	6918	-
	Total 1	3029	39	14079	47	19179	77

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1988 - 1991)

ST	ATE : BIH	AR				C = Ca	ses ; [) = Dea	ths
SI.	Affected		1988		1989		1990	•	1991
No.		С	D	C	D	С	D	С	D
140.	Diotriot			•					
1.	Patna	222	12	490	3	682	5	1296	32
2.	Nalanda	37	,	52	-	33	1	206	4
3.	Gaya		-	9	-	6		14	-
4.	Jahanabad (87)	- .		8	-	- 6	~	35	2
5.	Nawadah	_		-	-	4	-	35	2
6.	Aurangabad	-			••	1	-	13	-
7.	Bhojpur	17	-	169	-	77	5	69	1
8.	Rohtas	65	-	29	-	15	-	26	-
9.	Saran	350	-	552	3	1257	3	2105	29
10.	Siwan	200	_	418	2	1035	***	1216	2
111.		31	-	125	· -	314	4	756	6
12.		n 368	2	487	2	834	-	2018	20
13.	· ·		-	215	. 1	66	1	89	1
14.	Muzaffarpur	1264	6	3721	92	5434	56	8323	152
15.	Sitamarhi	. 1146	1	2157	32	2885	54	2477	44
16.	Vaishali	394	-	3047	175	9916	213	9658	281
17.		1211	35	2229	36	4593	36	4277	35
18.		908	43	1717	26	2787	41	3593	47
19.		1655	16	4287	49	9740	120	6932	118
20.		341	-	332	-	1270	-	1785	-
21.		86	3	177	18	224	6	483	9
22.		127	-	194	1	938	8	1308	2
23.		221	2	1238	13	1844	23	2435	30
24.		1762	2	1605	3	1642	-	1331	2
25.		674	1	785	-	833	3	950	3
26		879	-	1647		2990	3	2752	2
27		213	-	228	-	1050	. 3	1268.	.3
28		9	-	-	-	* 4	-	8	-
29		7078	-	4705	21	3357	4	2411	a
30	· ,	_	-		-	* -	1 -	3	-
31		355	-	280	-	813	-	1742	1
	Total	19639	123	30903	477.	54650	589	59614	834

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA 1992 - 1995

ST	ATE : BIH	IAR	C	= Case	es; D	= Deaths	s ; P =	Provisi	onal
SI.	Affected		1992		1993		1994		1995(P.
No.	District	С	D	С	D	С	D	С	D
1.	Patna	895	10	370	1	253	1	183	1
2.	Nalanda	214	6	97	2	57	1	39	1
3.	Gaya	3	-	1	-	2	-	2	-
4.	Jahanabad	30	-	36	-	51	-	18	2
5.	Nawadah	, 9	-	7	-	3	-	11	-
6.	Aurangabad	2	~	-	400	1	~	2	404
7.	Bhojpur	277	15	136	7	96	14	90	des
8.	Rohtas	6		3		-	-	1	-
9.	Saran	3526	81	1674	38	934	15	617	8
10.	Siwan	2133	3	1075	1	464	1	293	-
11.	Goopalganj	1652	9	1245	8	541	11	337	1
12.	East Champa	ran 6098	45	3471	17	1964	16	1881	12
13.	West Champa	aran 290	21	155	9	198	5	327	6
14.	Muzaffarpur	12421	438	6925	210	3183	121	2082	55
15.	Sitamarhi	2776	80	1315	22	724	24	510	11
16.	Vaishali	8758	258	3037	138	1788	76	1555	52
17.	Darbhanga	3935	57	3006	46	2061	14	2330	12
18.	Madhubani	3011	46	1729	35	1339	17	1312	27
19.	Samastipur	8608	243	6533	124	4032	50	3132	46
20.	Bhagalpur	2180	-	918	60	419	-	248	-
21.	Monghyr	481	3	296	1	147	-	48	-
22.	Khagaria(83)	2151	8	1130	40	751	2	769	-
23.	Begusarai	-2718	57	1197	31	653	12	602	13
24.	Purnea	1472	2	1093	2	875	2	410	, , - ,-
25.	Katihar	1530	3	1002	2	633	2	515	. 1
26.	Saharsa	3571	15	2036	6	1098	2	1125	5
27.	Madhepura	1314	1	932	-	699	1 mg	820	. 2
28.	Dumka	8	-	25	-	47	-	-	-
29.	Sahebganj	2454	12 -		6	1074	1	985	-
30.	Gooda -	3000	4	2518	-	304	2	235	-
31.	Suppaul		-	-	-	•	-	172	-
32.	Kishanganj	w .	- '	-	-	74	-	153	- 1
33.	Ararea	-	86	-	-	- 1	-	186	
34.	Banka	•	-	-	-	**	-	34	-
35.	Buxar	•	-	-	-	-	_	21	-
	Total	75523	1417	44155	706	24391	379	21045	259

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1977 - 1980)

ST	ATE : WEST BE	NG	AL			C = (Cases ;	D = De	eaths
SI. No.	Affected District	C	1977 D	С	1978 D	С	1979 D	C	1980 D
1.	Malda			-				295	4
2.	Murshidabad			-	-			14	-
3.	West Dinajpur			-	-			-	-
4.	24-Paraganas (N)			-	-			24	2
5.	24-Paragans (S)								
6.	Nadia			-	-			**	-
7.	Hooghly			•	-			-	-
8.	Burdwan			-	-			444	-
9.	Darjeeling			-	-			•	-
	Total 6	3*	-	-	•	71*	1*	333	6

^{*} Districtwise distribution not available

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1981 - 1984)

ST	ATE: WEST	BEN	GAL			C = Ca	ses ; D	= Dea	ths
SI. No.	Affected District	С	1981 D	С	1982 D	С	1983 D	С	1984 D
1.	Malda	304	_	515		357	-	479	-
2.	Murshidabad	564	5	490	-	907		667	-
3.	West Dinajpur	-	-	172	1	1375	7	3039	-
4. 5.	24-Paraganas (I 24-Paragans (S		- .	57	2	78	-	48	-
6.	Nadia	-	-		~	-	**	-	-
7.	Hooghly	-	-	-	-	-	-	-	-
8	Burdwan	-	-	-	-	-	_	-	-
9.	Darjeeling		-	-	-		-	-	-
	Total	917	5	1234	3	2717	7	4233	-

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1985 - 1987)

ST	ATE: WES	T BEN	GAL	C = Cases ; D = Deaths					
SI. No.	Affected District	С	1985 D	·c	1986 D	С	1987 D		
1.	Malda	560	-	1175	-	2013	1		
2.	Murshidabad	638		648	-	860	1		
3.	West Dinajpur	2960	4	1514	3	1341	··· .		
4.	24-Paraganas (N). 89	1	207	-	51	-		
5.	24-Paragans (S	5)		100	.20	103	4		
6.	Nadia	-	-	65	2	16	1		
7.	Hooghly	-	_	9	-	15	3		
8.	Burdwan	-	-		-	48	0+ (19 Sus)		
9.	Darjeeling	•		-	-	-	-		
	Total	4247	5	3718	25	4447	10		

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1988 - 1991)

ST	STATE: WEST BENGAL C = Cases; D = Deaths								ths
SI. No.	Affected District	С	1988 D	C	1989 D	С	1990 D	С	1991 D
1.	Malda	1294	-	1270	2	1024		526	-
2.	Murshidabad	816	-	669	-	760	1	745	-
3.	West Dinajpur	573	-	1078		654	-	322	-
4.	24-Paraganas (N	1) 35	-	296	12	371	3	244	-
5.	24-Paragans (S)	179	2	130	3 -	112	5	64	2
6.	Nadia	7	-	63	2	30	-	29	-
7.	Hooghly	18	-	6	-	32	1	2	-
8.	Burdwan	146	0+	61	1	28	2	17	1
			(3sus)						
9.	Darjeeling	-	-	-	-	26	4	81	-
	Total	3068	2	3573	20	3037	16	2030	3

DISTRICTWISE KALA - AZAR CASES AND DEATHS IN INDIA (1992 - 1995)

STATE: WEST BENGAL				C = Cases ; D = Deaths ; P = Provisional					
SI. No.	Affected District	С	1992 D	С	1993 D	С	1994 D	1: C	995(P) D
1.	Malda	300	-	267	-	84		73	-
2.	Murshidabad	465	-	397	1	426	1	578	6
3.	West Dinajpur	-	_		-	-		-	-
4.	24-Paraganas (N) 269	-	256	_	250		452	11
5.	24-Paragans (S)	78	2	73	2	133	2	136	1 .
6.	Nadia	12	-	3	-	5	-	15	-
7.	Hooghly	6	-	2	-	7	-	4	-
8.	Burdwan	1	-	4	-	2	-	-	-
9.	Dargelling	81	-	45	-	86	-	142	-
10.	Dinajpur (N)*	-	-	121	-	18	-	11	- 1
11.	Dinajpur (S)*	362**		130	-	138	-	101	-
	Total	1574	2	1298	3	1149	3	1552	18

^{*} Previously constituted West Dinajpur
** Including Dinajpur (North)

NOTES

NOTES

